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PHYSIOLOGICAL REVIEWS

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THE VISUAL CENTRES OF THE BRAIN AND THEIR CONNEXIONS

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In recent years a considerable amount of experimental work has been carried out with a view to establishing the finer details of the representation of the retina in the lower and higher visual centres of the brain and to tracing the route by which retinal impulses are conveyed to their destination, and some of the earlier conceptions regarding the diencephalic and mesencephalic terminations of the optic tract have been shown to be erroneous. This work has been necessitated partly by the requirements of experimental psychologists who have made use of visual reactions in their studies of the process of learning and habit formation. The effect of light stimuli on gonadal and other activities has further aroused interest in the anatomical basis of visual reactions.

The structural organization of the retina. Since the classical studies by Cajal, the neuronal structure of the retina in the higher mammals had not received further attention until the histological investigations of Poliak in 1936 (75). Using the Golgi technique, this author made a number of original observations on the conducting units of the retina in monkeys and apes which clearly have an important bearing on the physiology of vision. The most pertinent of these observations are briefly as follows. While the rod elements are all related to the diffuse type of bipolar cell (i.e., several rods are related to one bipolar cell and there is some degree of overlap), the cones are synaptically related to both the diffuse and the individual types of bipolar cell. In the case of the latter, the relationship is mostly monosynaptic, particularly in the fovea and macula, each cone pedicle directly touching the dendritic tuft of a single bipolar cell. In the case of the diffuse varieties, even in the macula, each bipolar cell is related to several cones (and simultaneously to a group of rods). Poliak recognizes four varieties of the diffuse or polysynaptic type of bipolar cell. One of these, the *d* bipolar, corresponds to Cajal's "rod bipolar," but it is found to be related synaptically to cones as well as rods, so that Cajal's terminology is not altogether apt. Attention is also drawn to the fact that not only is the dendritic tuft of each *d* bipolar related to a compact group of photoreceptors, but the tufts spread over the territories belonging to those of adjoining bipolar cells of all varieties, both diffuse and individual. The individual bipolar may be appropriately termed a "cone bipolar," since this variety is related to cones alone. As a rule there is no reciprocal overlapping between the dendrites of adjoining cone bipolars, particularly in the central region of the retina, and here most of them are related individually to single cones. In the more peripheral parts of the retina, however, a limited degree of overlapping may occur. A consideration of the disposition of the diffuse bipolar cells indicates that even at this neural

level in the visual path (and still more so at the ganglion cell level) the two retinal mechanisms related respectively to photopic and scotopic vision are by no means completely separated from each other. Another point which emerges from this histological study is that, although it is not possible by Golgi impregnation to demonstrate more than a single morphological type of cone, the several varieties of bipolar cells may constitute the analysers postulated by the three-component hypothesis of colour vision of Young, Helmholz and Maxwell.

As with the bipolar cells, Poliak found in the higher primates two groups of ganglion cells, the diffuse and the individual. Each individual ganglion cell (also called a "midget ganglion cell" because of its small size) effects a synaptic contact with a single individual or cone bipolar cell, and is therefore related to a single cone. Thus, as Poliak emphasizes, there is indeed an anatomical basis for a one-to-one relationship between the neurones of different levels in the retina. However, it seems that some individual ganglion cells may at the same time be in synaptic contact with bipolar cells of the diffuse type. The diffuse ganglion cells have relatively long or numerous dendrites, with a considerable reciprocal overlapping of the territories of adjacent cells.

Horizontal cells are present in the inner nuclear layer and are most numerous in the macula and fovea. Their axones are disposed in no particular direction and each cell brings into reciprocal relationship one group of cones (up to 15 in the macula) with another and larger group of cones and possibly also with a group of rods. The "amacrine" cells are difficult to evaluate, for the term has been applied to glial as well as to nervous elements. Some appear to be associational cells with a horizontal axone running in the inner molecular layer for some distance; others appear to be merely ganglion cells displaced into the inner nuclear layer.

In relation to the purely histological evidence for a point-to-point projection system from the retina to the lower visual centres, may be mentioned the oscillographic observations of Hartline (43). This author investigated the receptive fields of individual optic fibres in certain cold-blooded vertebrates (frog and alligator) by recording the action potentials in single fibres in response to the illumination of various parts of the retina. It was concluded from these experiments that the receptive field of an optic nerve fibre covers an area much greater than that occupied by a single rod or cone; that a retinal ganglion cell can therefore receive excitatory influences over many convergent pathways; and that the axone of a ganglion cell is simply the final common path for nervous activity originating in many sensory elements. However, though this may be the case with many optic fibres (as is certainly affirmed by the histological observations of Poliak), it does not seem possible on the basis of Hartline's work to exclude other types of fibre with much more restricted receptive fields. In any case, of course, Hartline's studies were not concerned directly with the mammalian retina.

Optic nerve. Contrary to some statements, observations of Bruesch and Arey (18) have shown that in most mammals including man and other primates all the fibres of the optic nerve are myelinated. In some lower mammals, however, a considerable proportion may remain unmyelinated (e.g., 33 per cent

in the opossum and 44 per cent in the bat). These authors have also shown that the number of fibres in the optic nerve shows considerable variation in mammals, partly (but by no means consistently) related to the size of the eye. The following computations were made:

Opossum	82,000
Bat (species not indicated)	7,000
Dog	146,000
Cat	118,000
Rabbit	265,000
Guinea pig	126,000
Monkey	1,208,000
Man	565,000–1,140,000

Perhaps the most striking feature of this table is the fact that in the rabbit (an animal in which the panoramic type of vision predominates) the optic nerve fibres are more than twice as many as those of a cat (an animal in which visual acuity is commonly regarded as relatively well developed and in which the optic nerve is actually larger in diameter). It is also somewhat surprising to find that a monkey has more optic nerve fibres than man; on the other hand, it is a fact that the lower and higher visual centres of the brain of some species of monkey do show a distinctly higher degree of structural differentiation. The numerical relation of optic nerve fibres to retinal ganglion cells has been recently studied by Arey (2). Whereas previous observations had suggested that the number of ganglion cells is much in excess of the optic fibres (indicating that many of the ganglion cells do not send processes back to the brain), Arey found that in the dog there is good reason to believe that a simple 1:1 ratio exists. It is probable, indeed, that in earlier work the numerous glial cells of the ganglion cell layer of the retina had not been excluded from ganglion cell counts.

The fibres of the optic nerve show a tendency to a topographical grouping in relation to the retinal site of their origin, but it appears that this localization is not very sharply defined. Brouwer and Zeeman (17), on the basis of Marchi preparations, found in the monkey that the fibres from the upper half of the retina are situated above those from the lower, and that the temporal fibres lie lateral and the nasal medial. On the other hand, they concluded that the macular fibres do not show a very exact localization; near the eye they occupy a lateral position in the optic nerve, but as they approach the chiasma they come to be more centrally placed. It is evident, therefore, that some regrouping of fibres occurs in the antero-posterior course of the optic nerve.

The optic nerve fibres also show a differentiation according to size and rate of conduction. Curiously enough, the anatomical details of this differentiation have hitherto received very little attention, though appropriately stained sections of the optic nerve demonstrate clearly enough that there is a considerable range in the calibre of the fibres, and this range apparently varies in different species of mammal. The problem has however been approached from the electro-physiological side by Bishop (10). This author found evidence for the existence in the frog's optic nerve of three rather distinct groups of fibre, differing in conduction rate, threshold to electrical stimulation, and other physio-

logical properties. In the rabbit's optic nerve oscillographic records were found to be similar to those of the frog, except that one wave (the *C* wave) appeared to be lacking or else extremely low. The first group of fibres in the optic nerve corresponds in conduction rate not to the first group in most general sensory nerves, but to a slower element. In the rabbit, for example, the rate is somewhat faster than that characteristic of the sensory B group of the saphenous nerve, which mediates pain, temperature and vaso-motor impulses. The second group does not seem to correspond with any well-defined group in other types of sensory nerve. Lastly, it is supposed that the third group (in the frog) may consist of efferent fibres. The significance of these different types of fibre in the optic nerve is unknown, except that (in mammals) the larger fibres are mainly destined for the lateral geniculate body and the finer fibres for the mid-brain. Bishop remarks that by analogy with the sensory nerves "one might anticipate that the larger fibres of the optic nerve would mediate that aspect of vision concerned with spatial discrimination or form, while the smaller fibres would be concerned with the quantitative factor of intensity."

The presence of centrifugal fibres in the optic nerve, passing to the retina, has been a source of dispute in the past. Several authorities have expressed doubt as to whether such fibres exist in mammals and this doubt now seems to be fully confirmed by the study of the fibre content of optic nerves after enucleation of the eye. Bodian (13), for example, reports that, in the opossum, no normal nerve fibres are to be found in reduced silver preparations of optic nerves 10 weeks after removal of one or both eyes.

Previous investigations seem to have established the fact that in certain lower vertebrates efferent fibres to the retina do exist in the optic nerve. For example, Arey (1) brought forward experimental evidence to show that in *Ameirus* there are functional efferent nerves which in some manner control the photomechanical responses of the rods, cones and retinal pigment. He suggested that the efferent impulses do not directly stimulate the motility of the retinal elements, but that they have an indirect action by counteracting the tonic inhibition exerted by the ciliary nerves. However, in certain other fishes, and also in frogs, no such function could be demonstrated, and it remains possible, therefore, that the mechanism is peculiar to *Ameirus*.

Optic chiasma. It has not infrequently been stated that a partial decussation of the retinal fibres in the chiasma only occurs in man and in the higher mammals—in lower mammals the decussation was presumed to be complete. It is probably true to say, however, that in no mammal is this the case. In the lowly opossum, for example, as many as one-fifth of the retinal fibres remain uncrossed (Bodian, 13), and in the ferret about one-third (Jefferson, 47). It has also been supposed that the development of only a partial decussation of the optic nerves is related to the development of a fovea centralis in the retina, and to the development of a precise type of conjugate movement. This again seems to have been a misconception, for Detwiler (33) has pointed out that a fovea is found in fishes and Lacertilia in which the decussation of retinal fibres is complete, and, moreover, the complete decussation in the chameleon is hardly consonant with the

suggestion that a partial crossing is a necessary condition for a wider range and a greater exactitude in conjugate movements of the eye.

The proportion of optic fibres which cross in the optic chiasma in higher mammals has not been established by exact computational methods. It is known from experimental evidence that in monkeys all retinal fibres from the temporal side of a vertical axis passing through the centre of the macula pass to the ipsilateral optic tract, while those from the nasal side undergo a decussation. Indirect observations on the monkey (based on the relative number of cells in the geniculate body receiving, respectively, crossed and uncrossed fibres) have suggested that in this animal (so far as the geniculate body is concerned) about 40 per cent of the fibres in each optic nerve remain uncrossed (Clark, 28).

The intimate topographical relation between the optic chiasma and certain hypothalamic commissures in this region make it very difficult in normal preparations to decipher the precise course of the retinal fibres here. From time to time statements have been made with reference to optic fibres that are supposed to leave the chiasma and terminate in the grey matter of the hypothalamus. Experimental studies, however, have discounted such a conclusion. In a critical examination of the optic tract system of the ferret, Jefferson (47) showed that it is very easy to misinterpret the appearance of normal Weigert and silver preparations. For one thing, the fibres of the supra-optic commissures (which probably have nothing to do with visual functions) are not always distinguishable from retinal fibres. Also, in the chiasma the decussating fibres may run an aberrant course which can be very misleading to the casual observer. Some fibres, for example, may run up from the chiasma towards the wall of the 3rd ventricle and then loop down again to rejoin the optic tract on the opposite side. It seems certain that such aberrant fibres have been mistaken in the past for terminal optic fibres related to the hypothalamus, simply because they have not been traced in appropriately stained material by the study of serial sections. The discrete median "dorsale hypothalamische Wurzel" of the optic tract described by Frey (36) as entering the tuber cinereum from the dorsal and caudal aspect of the chiasma is evidently based upon an error of interpretation of a different category, for he seems to have mistaken the appearance of a tangential section of the dorsal convexity of the chiasma for a median tract.

Optic tract. All the experimental evidence at present available goes to show that the homolateral and heterolateral fibres from corresponding areas of the retinae are intimately and evenly mingled with each other in the optic tract. Even in man and other higher primates there is no indication of a stratification such as is found in the terminations of these sets of fibres in the main lower visual centre—the lateral geniculate body. Thus, some weeks after the section of one optic nerve in a monkey, the remaining normal fibres are found to be disposed perfectly evenly throughout the optic tracts (Clark, 28). It follows, therefore, that the crossed and uncrossed fibres only become disentangled and separated from each other inside the geniculate body. On the other hand, it has been established for some time that, at least in the primates, the fibres from the different quadrants of each retina retain a fairly definite segregation in the

optic tract, in the sense that those from the upper quadrants are disposed dorsally, and those from the lower quadrants ventrally. The macular fibres occupy the central part of the tract, extending over a fairly wide area dorso-laterally.

Accessory optic tracts. Besides the main optic tract which proceeds back from the chiasma to the lateral geniculate body and the mid-brain, two accessory tracts have been described which, leaving the main tract, terminate in the region of the subthalamus. The anterior accessory optic tract (of Bochenek) is commonly described as penetrating the cerebral peduncle to end in the subthalamic nucleus (Corpus Luysii), while some of its fibres may reach the same nucleus more directly by passing over the dorsal surface of the cerebral peduncle. The presence of this tract was affirmed in Marchi preparations of the rabbit's brain by Pavlow (71) and Loepp (56). However, there remains grave doubt whether a tract with such connexions really exists, for other interpretations of this bundle of fibres have been put forward. For example, Kosaka and Hiraiwa (49) believed it to be merely an aberrant bundle which subsequently rejoins the main optic tract. Lashley (51) identified the tract in the rat's brain, but found that it divides into two branches, of which one rejoins the main optic tract while the other joins the posterior accessory optic tract. The previous work of Overbosch (69) had also suggested that the anterior tract might be simply an aberrant part of the posterior tract. The apparent absence of an anterior accessory optic tract has been recorded in the opossum (Bodian, 13) in the cat (Barris *et al.*, 7) and in the ferret (Jefferson, 47). Indeed, whatever may be the case in the rat and rabbit, the opinion is expressed by Barris *et al.* that the tract does not exist in higher mammals. In a more recent study, Gillilan (37) affirms the existence of the tract (as well as its termination in the subthalamic nucleus) in bats, insectivores and rodents, this conclusion being partly based on the study of Marchi preparations from experimental material. Probably, however, before these conclusions are finally accepted in the face of the negative observations recorded by many careful workers, it will be necessary to employ an experimental technique which is more refined than the Marchi method. Incidentally, it may be noted that the fibres of the so-called anterior accessory optic tract are extremely fine, so that it is by no means easy to follow them accurately with Marchi staining.

The posterior accessory optic tract is described as leaving the main optic tract close to the ventral pole of the lateral geniculate body, crossing obliquely over the cerebral peduncle (forming here the tractus peduncularis transversus), and entering the interpeduncular space. Here it terminates in a collection of cells, the nucleus opticus tegmenti, which is interposed between the mammillary body and the cerebral peduncle, and also (probably) in the medial extremity of the substantia nigra. There can be little doubt that the posterior accessory optic tract exists in some lower mammals. Since it was first described by Gudden (41), its existence has been confirmed experimentally in the rabbit by Pavlow (71), Loepp (56), in the guinea pig by Wallenberg (86), Castaldi (20) and Frey (36), in the rat by Clark (24), Lashley (51), Chang (21) and Tsang (82), in the opossum by Bodian (13), and in the phalanger by Packer (70). But there remains con-

siderable doubt whether the tract is present in the brain of higher mammals. Thus Barris *et al.* (7) were unable to find it in the cat, or Jefferson (47) in the ferret. On the other hand, Gillilan (37) has more recently reached the conclusion that it is present in the cat and the monkey, but it must be admitted that the evidence of this author as presented is perhaps not altogether convincing. Lastly, it is wēll to point out that statements made in the past by some comparative neurologists on the course and distribution of the accessory optic tracts have evidently been based entirely on the examination of normal histological material. Such material, however, is readily liable to misinterpretation, for it does not permit of the assumption that fibres seen in the position of the so-called accessory optic tracts are really retinal in their origin.

The functional significance of the accessory optic tracts when they are present (e.g., in rodents) is quite unknown. Lashley (53) concluded from his experiments that they are not adequate for the formation of visual habits based on brightness discrimination. Attempts were made by Clark, McKeown and Zuckerman (31) to determine whether they might be concerned with the gonadal response shown to retinal stimulation in ferrets. It was found that bilateral section of the optic tracts at the ventral pole of the lateral geniculate body made no difference to this response, which seemed to indicate that it might be mediated by the accessory tracts. However, the evidence was inconclusive, for careful examination of serial sections showed that in all the experiments a very few optic fibres (passing either to the lateral geniculate body or to the mid-brain) had escaped injury on one side. This problem, therefore, requires a re-investigation.

The tectal connexions of the optic tract. Most of the retinal fibres which reach the roof of the mid-brain (at least in most mammals) end in the superior colliculus. Marchi preparations following section of the optic nerve have shown that these collicular fibres can be followed into the layer of white matter which is termed the stratum opticum. Hereafter the fibres in general lose their myelin sheaths, so that the actual site of their terminal arborization cannot be followed with certainty either in Weigert preparations, or by the Marchi technique in which degenerating myelinated fibres are stained differentially with osmic acid. By the latter method, indeed, some observers have followed mesencephalic fibres of the optic tract from the stratum opticum into a layer of cells lying immediately superficial to it, the stratum griseum superficiale, and from silver-impregnated sections of normal material a similar conclusion can be drawn. Further, it has been shown by Tsang (82) that the cells of the stratum undergo a pronounced degree of atrophy several months after section of the optic nerves in young rats. The precise site of termination of optic fibres in the tectum has recently been established by studying silver-impregnated material from animals in which one optic nerve had been sectioned 48 and 72 hours before death (Jefferson, 47). In these sections it was found that the terminal ramifications of the mesencephalic retinal fibres had undergone a very distinct fragmentation and granulation, and these degenerative changes were found to extend not only throughout the stratum griseum superficiale but also in the most superficial zone of the superior colliculus, i.e., the stratum zonale. It may now be accepted,

therefore, that retinal fibres run into the superior colliculus to form a layer of white matter, the stratum opticum, and terminate in all that part of the colliculus which lies superficial to this layer.

The localization of retinal fibres in the superior colliculus was studied in the rabbit by Overbosch (69) and in the rat by Lashley (51). In these animals the localization is of a fairly precise nature, to the extent that the lower temporal fibres of the retina are projected on to the antero-medial quadrant of the colliculus, the upper temporal to the antero-lateral quadrant, the inferior nasal to the postero-medial, and the superior nasal to the postero-lateral. Bodian (13) has reported a similar localization in the opossum. The course and ending of the mesencephalic fibres suggest that in the monkey also there is a localization of the different parts of the retina over the surface of the colliculus. It seems that in many mammals few if any uncrossed fibres from the retina reach the superior colliculus or, indeed, any part of the tectum. Certainly this appears to be the case with the rat, rabbit and ferret, and, indeed, such a conclusion is consonant with the conception that the mesencephalic tectum is the most primitive of the lower visual centres and that in lower vertebrates the decussation of optic fibres in the chiasma is primitively complete. On the other hand a few retinal fibres have been described as passing to the homolateral colliculus by some observers, e.g., Barris, Ingram and Ranson (7) in the cat and Bodian (13) in the opossum, but these are far less numerous than the fibres which pass to the opposite colliculus.

In their experiments on the monkey Brouwer and Zeeman (17) had concluded on the basis of Marchi material that no fibres from the macula reach the superior colliculus, and this was rather a surprising result since it is supposed that pupillary reactions are most lively when light falls on the macula. It was indeed concluded that if fibres concerned in the reflex movements of the pupil take origin in the macula they must be unmyelinated (for such fibres would not of course be evident in Marchi preparations). However, as we have already noted, the observations of Bruesch and Arey have demonstrated that in the monkey there are no unmyelinated fibres in the optic nerves. In recent years the explanation of this discrepancy has become apparent. For a long time comparative anatomists have described in the brain of lower mammals a rather well-circumscribed oval group of small cells situated at the dorsal surface of the tectothalamic junction, immediately under cover of the anterior and lateral margin of the superior colliculus. This group of cells is now termed the pretectal nucleus. By some authors the element has been regarded as the homologue of the pulvinar in the primates, but this identification is certainly erroneous, for the pulvinar is nothing more than the posterior part of the lateral nucleus of the thalamus which, in higher mammals, has become pushed backwards to form a prominent eminence by the expansion of the main part of the lateral nucleus which lies in front of it (Clark, 23). Lashley (51), in Marchi material, found degeneration within the pretectal nucleus following section of an optic nerve, and convincing evidence has since been adduced to show that the pretectal nucleus is an essential element in the pathway of the reflex constriction of the pupil.

The course of the pupillo-constrictor fibres has been followed in the cat by Hare, Magoun and Ranson (42) by noting the pupillary reaction which occurs on the electrical stimulation of successive points along the optic tract with a stereotaxic apparatus. By previous sectioning of the optic nerves, allowing time for the retinal fibres to undergo degeneration, they have shown that the pupillo-constrictor fibres have their first synapse in the pretectal nucleus. From here the impulses are relayed by fibres which mostly cross in the posterior commissure to the oculomotor nucleus of the opposite side, though some pass to the ipsilateral nucleus. Pupillo-constrictor fibres of cortical origin extend from a cortical area adjacent to the visual cortex directly to the pretectal region. Hence lesions of the optic tract may abolish pupillary reaction to light while maintaining the integrity of the cortical conveyance reaction. In the monkey, the same disposition was found. Light reflex fibres pass toward the superior colliculus. They do not enter the latter, however, but turn rostrally and medially into the pretectal region. Thence the path passes to the oculomotor nucleus by fibres which may decussate in the posterior commissure and also ventral to the cerebral aqueduct.

If the light reflex of the pupil is mediated by the pretectal nucleus which lies under cover of the margin of the superior colliculus, it remains to consider what functions are subserved by the superior colliculus itself in virtue of the termination in its superficial layers of retinal fibres. In the monkey it is commonly stated that relatively few optic fibres reach the superior colliculus and that in man the retinal connexions are even more insignificant. However, it seems that such statements do not depend on any method of direct computation but are mainly based on the general impression gained by the study of sections of Marchi material. Marchi sections, however, are easily open to misinterpretation in this kind of problem. For one thing, the optic fibres which reach the mid-brain are more finely medullated than those which terminate in the lateral geniculate body; thus the Marchi staining will be relatively much less intense in the mesencephalic fibres, suggesting to the uncritical eye that they are few in number. In lower mammals large numbers of retinal fibres certainly end in the colliculus and in correlation with this it has been shown experimentally that in these animals the superior colliculus provides an integrating mechanism of considerable complexity in relation to visual impulses. It has been shown, for example, that rodents with no visual cortex at all can discriminate light intensity and can also judge the position and distance of an object (Ten Cate and van Herk, 81; Lashley, 50). Under similar conditions dogs can also retain their powers of discrimination for different intensities of light, though in these animals the recognition of the position and distance of an object is a cortical function (Marquis and Hilyard, 59; Marquis, 57). Even in monkeys a conditioned response which has been established to a light stimulus may be retained after the entire removal of the visual cortex (Marquis and Hilyard, 60), whence it may be assumed that this function is mediated by the mid-brain. On the other hand, destruction of the visual cortex in man leads to complete and permanent blindness. Marquis (58) has drawn attention to these differences in the results

following removal of the visual cortex in different species, and has shown that it represents a phylogenetic sequence in the gradual "corticalization" of visual functions in the brain.

The lateral geniculate body. The lateral geniculate body is to be regarded as one of the sensory nuclei of the thalamus, and in lower mammals it may be only imperfectly demarcated from the main ventral nucleus of the thalamus in which fibres of the fillet system terminate. In primitive mammals, also, the visual fibres which end in the lateral geniculate body are probably entirely collaterals of the main optic tract fibres which are passing by on their way to the mid-brain. In other words, primitively the lateral geniculate body appears to be functionally subordinate to the tectum as a visual centre. If mammals are arranged in a phylogenetic series it is possible to demonstrate that, in an ascending scale, proportionately more and more direct optic fibres end in the lateral geniculate body, and, since the latter is essentially a relay station for the projection of retinal impulses on to the cortex, this is an anatomical reflexion of the increasing dominance of the cortical control of visual functions in the evolutionary series.

If the lateral geniculate body of a lower mammal (e.g., a rabbit) is viewed in a transverse section of the thalamus, it appears to consist of two parts—a larger portion with relatively large cells, the dorsal nucleus of the lateral geniculate body, and a ventral segment containing small cells, the so-called ventral nucleus. In Weigert and silver sections, bundles of the optic tract can be seen to run through the ventral nucleus on their way to the dorsal nucleus, and a number of fibres appear to end in the former. Indeed, since Cajal (19) described and figured collaterals from the optic tract fibres ending in the ventral nucleus, this element has been for a long time widely accepted as one of the lower visual centres. Moreover, it has been regarded as a more primitive diencephalic visual centre than the dorsal nucleus, for it is but a lateral extension of the lower functional levels of the subthalamus, and it shows a gradual retrogression in an ascending phylogenetic series. However, doubt has been expressed in recent years as to whether the ventral nucleus in reality has anything to do with vision. The difficulty of arriving at a decision depends on the fact that in this region the fibres of the optic tract are intermingled to a considerable extent with other fibre systems, so that, however persuasive the appearance of a normal Weigert or silver preparation may be, it is not possible with such material to affirm with certainty that *retinal* fibres end in the ventral nucleus of the lateral geniculate body. Certainly a large proportion of the optic tract fibres here are merely fibres of passage directed towards the dorsal nucleus. The study of Marchi degeneration after section of one optic nerve leads to negative results in this problem, for the osmic staining appears to be confined to the fasciculi of fibres of passage, while the ground-substance of the nucleus remains free of deposit. This is in marked contrast with the dorsal nucleus of the lateral geniculate body, the ground-substance of which is richly sprinkled with black osmic granules in similar experiments. There remains the possibility, however, that fine unmyelinated collaterals of the optic fibres (which would not be demonstrable by the Marchi technique) terminate in the ventral

nucleus. On the basis of Golgi material of the cat's brain, O'Leary (66) has recently described very fine fibres which leave the position of the optic tract to end in the nucleus, but, here again, it has yet to be proved that these fine fibres have their origin in the retina. The study of silver impregnated material in which the optic tract has undergone complete degeneration after section of the optic nerves provides no further positive evidence, for it has been found that in the ferret such material shows no diminution in the fine neuropil pervading the ventral nucleus as compared with the normal side. Lastly it has been observed by Tsang (82) that, after unilateral or bilateral extirpation of the eyes in rats, there is never atrophy of the cells of the ventral nucleus of the lateral geniculate body, however long the period of degeneration. On the other hand, after similar operative procedures the cells of the dorsal (or main) nucleus become shrunken, rounded and embryonic in form, and with fewer processes than normal.

The main part of the lateral geniculate body[1] (the dorsal nucleus as it is commonly termed by comparative anatomists) is by far the most important of the lower visual centres in higher mammals, and particularly in man. In a phylogenetic series of mammals it undergoes an increasing cyto-architectural differentiation, and also shows a progressive tendency to become clearly and sharply laminated. Such a laminar arrangement of the geniculate cells may be seen in an incipient form even in the brains of primitive mammals; in higher mammals it becomes very distinct, and it is now known that this lamination is primarily related to the termination of crossed and uncrossed fibres. But the number of cell-layers varies in different groups. In the cat, for example, there are three main laminae, and of these the middle lamina receives uncrossed retinal fibres while the other two are related to crossed fibres. In all the higher primates (including man, apes and Old World monkeys) there are six laminae, three of which are connected with each retina.

The precise mode of termination of retinal fibres in the lateral geniculate body is a matter of considerable interest from the point of view of visual physiology. First of all it may be mentioned that, at least in lower mammals (and possibly in man also), mesencephalic fibres of the optic tract may give off collaterals which terminate in the lateral geniculate body. This is the case, for example, in the cat, and it has been emphasized that it is of some functional significance that one and the same fibre from the retina may mediate impulses to the tectum of the mid-brain, and also the visual cortex by way of the lateral geniculate body (Barris, Ingram and Ranson, 7).

The earlier work of Brouwer (15) and Brouwer and Zeeman (17) had shown that a localization of quite a precise type is present in the lateral geniculate body. In the rabbit the localization is such that the upper half of the retina projects on to the lower half of the lateral geniculate body and *vice versa*. In the monkey, following the rotation of the lateral geniculate body which has occurred during its development, the projection is rather different, for now the fibres from the upper half of the retina end in the medial half of the nucleus, while those from

[1] Hereafter the term *lateral geniculate body* will be used entirely in reference to the main or dorsal nucleus.

the lower half of the retina terminate laterally. In the monkey, also, the macula has quite a definite representation of its own, and this occupies a relatively large area which appears as a median sector of the geniculate body.

In 1934 Clark and Penman (30) carried out experiments on monkeys similar to those of Brouwer and Zeeman (by the production of small lesions in different parts of the retina), but using instead of the Marchi technique a more precise method for localizing in the lateral geniculate body the site of termination of the retinal fibres which had been involved in the lesions. This method depends on the fact that a very marked transneuronal degeneration occurs in the lateral geniculate body of monkeys after the interruption of optic fibres. The reason for this type of degeneration in this particular nucleus is not clear, unless indeed it is an indication of the extreme specificity of function of the cells of the geniculate body to the extent that they undergo rapid atrophy if they are not activated by the impulses which normally have their origin in the retina. In some lower mammals which have been investigated by the present writer (e.g., the rat and ferret) such distinct transneuronal degeneration does not occur, and in the cat it seems to be relatively slight, the changes in the cells "amounting to no more than a diminution of the amount of cytoplasm" (Barris, 5). In the monkey (and in man also) it is so marked as to allow a very precise determination of the position of any group of cells in the geniculate body which receives fibres from a small area of the retina.

By making use of this phenomenon of transneuronal degeneration it was possible to demonstrate that the retinal projection on to the lateral geniculate body in monkeys is even more precise than had been supposed. Indeed, practically speaking, the projection approximates to a point-to-point representation, for it is possible to map out the whole of the lateral geniculate body in terms of small and discrete retinal areas. The macular area of the retina was found to be limited to the caudal two-thirds of the nucleus, occupying a median sector which widens in a caudal direction at the posterior extremity where it covers the whole width of the nucleus. Various observations have also indicated that the localization of the macular and peripheral areas of the retina in the nucleus in man, anthropoid apes and monkeys is of an equivalent order (Balado and Franke, 3, 4; Brouwer, 16; Poliak and Hayashi, 76). The transneuronal experiments also demonstrated some interesting features with regard to the termination of crossed and uncrossed fibres in the geniculate body, and in order to give an account of this it is necessary to refer to the structure of the geniculate nucleus in man and monkey.

In the higher primates the lateral geniculate body is composed of six distinct and clearly demarcated cell laminae, separated by intervening layers of white matter. The two most superficial laminae are composed of relatively large cells, and the remaining four of closely-packed smaller or medium-sized cells. For convenience of description the layers may be numbered 1 to 6 from the surface inwards. Many years ago Minkowski (64) found that in a monkey in which one optic nerve had been sectioned some time before death, the lateral geniculate body of the opposite side showed a pronounced atrophy of the cells forming

layers 1, 4 and 6, while on the same side layers 2, 3 and 5 had been similarly involved. In other words, it appeared that crossed fibres of the optic tract terminate in one set of cell laminae (1, 4 and 6) while the uncrossed fibres end in the other set (2, 3 and 5).

Clark and Penman found in their experiments that the smallest retinal lesion which produces detectable changes in the lateral geniculate body nerve cells always leads to a zone of atrophy in the form of a band affecting all three of the corresponding cell laminae of the lateral geniculate body. In other words, this is the receptive unit of the lateral geniculate body in respect of each retina; these units altogether may be described as a series of narrow bands radiating from the region of the hilum towards the convex periphery of the nucleus, involving the three laminae related to the retina of one or other eye.

This arrangement is susceptible of two possible interpretations. Either *1*, each individual fibre from the retina (the axonal process of a single ganglion cell) terminates by a series of arborizations about cells in all the three laminae concerned, or *2*, the conducting unit of the optic nerve in respect of the lateral geniculate body is a unit of three fibres each passing to a separate lamina. The first alternative is almost certainly incorrect, for a careful study of silver-impregnated material fails to show any indication of the division of individual optic fibres, either in their course through the optic nerve and tract, or at their point of entry into the lateral geniculate body. The second alternative, it has been suggested (Clark, 27), may have a relation to the Young-Helmholtz theory of colour vision, for this theory involved the assumption of three types of sensory receptor in the retina and three sets of optic nerve fibres connecting them with the brain. In other words, it seems possible that the three laminae of the geniculate body related to each retina may be concerned with the three fundamental colour sensations postulated by the trichromatic theory of colour vision—red, green and violet.

Such a conclusion, of course, must remain entirely speculative in the absence of direct evidence. It may be noted that a recent study of the lateral geniculate body in the New World monkeys (Clark, 29) has failed to show any correlation between the degree of visible lamination and the differentiation of colour-vision as determined experimentally. It is true that in *Cebus*, which according to the evidence recorded by Grether (40) possesses dichromatic vision, the lamination is by no means so clear-cut as it is in *Macaca*. On the other hand, in the spider monkey (in which it appears that colour discrimination is fully equal to that of the Old World monkeys) the small-celled element of the lateral geniculate body shows no visible lamination. It is pointed out, however, that there may be a functional lamination which is not visibly manifest in the nucleus, and the existence of which could only be determined by the study of transneuronal degeneration following the section of one optic nerve.

It is of some importance to determine whether any mechanism exists in the lateral geniculate body which could provide the anatomical basis for a fusion at this level of crossed and uncrossed retinal impulses. It is well known that

small lesions in the lateral geniculate body lead to small, bilateral scotomata. But it is hardly possible that even the smallest lesions which can lead to a demonstrable scotoma will be confined to one cell-lamina only, and, even were this the case, it must inevitably involve optic fibres which penetrate the lamina to reach the next adjacent lamina. Henschen (45), on the basis of Golgi studies of the human geniculate body, concluded that intercalated elements are present through which the several cellular laminae are brought into functional relation with each other. However, Taboada (79) maintains that the diverse types of cell described by Henschen really represent different varieties of a single type, the differences in appearance being entirely due to the topographical position in which each cell finds itself. The same author has also established that, in the monkey, the dendritic processes of the geniculate cells are confined in their distribution to the individual lamina in which the cells are situated. In spite of the pronouncements to be found in the earlier work of neurohistologists, the precision with which transneuronal atrophy following section of one optic nerve is limited to one set of laminae only suggests a very high degree of functional independence. Further, the fact that complete ablation of the visual cortex leads to a total atrophy of all the cells of the large- and small-celled laminae of the lateral geniculate body seems to decide finally against the existence in these laminae of any intercalated neuronal elements whose functions might be presumed to link them up with each other. In other words, it is highly probable on anatomical grounds alone that crossed and uncrossed visual impressions remain isolated from each other in the lateral geniculate body of higher mammals, so that fusion can only occur in the visual cortex. A study of the electrical activity following optic nerve stimuli provides support for this conclusion, for, leading off from the geniculate body of one side (in the cat) while stimulating simultaneously or in succession both optic nerves with single shocks, Bishop and O'Leary (11) found no evidence of any phenomena of interference or facilitation which might suggest fusion of retinal images at the geniculate level. On the other hand, it had previously been reported by Marshall and Talbot (61) that facilitation can occur at the cortical level. Further findings from the oscillographic studies of Bishop and O'Leary may be mentioned here. In correlation with the histological evidence that, generally speaking, the finer fibres of the optic tract run to the mid-brain, while the coarser fibres end in the geniculate body, it was found that impulses reach the latter more rapidly. Indeed, it appears that, allowing for the conduction and synapse time in the pathways to the optic centres, then of two impulses started in parallel fibres of the optic nerve at the retina, the one passing to the cortex can be relayed down to the superior colliculus before the other has reached the colliculus directly. According to the authors, it would then be possible on physiological grounds for an animal to recognize a sensory stimulus in a shorter time than it could respond to it reflexly, i.e., there would be time for a message from the cortex to reach the reflex centres for regulation of the response before the reflex centres were activated directly by the same stimulus. The experiments further suggested that the majority of the fibres which activate the cortex do not activate the superior colliculus, and *vice versa*.

It is of considerable morphological interest to note again that, while the crossed and uncrossed retinal fibres terminate in different sets of laminae in the lateral geniculate body, they show no tendency to assume a laminar arrangement as they approach the latter in the optic tract. On the contrary, in the tract the crossed and uncrossed fibres are intimately intermingled. It thus appears that there is no question of the laminar arrangement of cells being secondarily imposed on the lateral geniculate body by the disposition in which the retinal fibres find themselves on their arrival at the end of the optic tract. On the contrary, the optic tract fibres penetrate the anterior and ventral aspect of the geniculate body and may pass through several layers of cells before they reach their own particular lamina.

The relation of the laminae in the lateral geniculate body to crossed and uncrossed optic fibres has been shown to be the same in man as in the monkey (Clark, 25; Hechst, 44; Barris, 5). In the cat, the nucleus consists of three main cellular laminae (of which the most superficial is sometimes subdivided topographically into two). Barris (5) has confirmed an earlier observation of Minkowski (63) that crossed retinal fibres terminate in the most superficial and the deepest of these layers, while the intermediate lamina receives uncrossed fibres.

While it is certain that in mammals such as man, monkeys and cats in which the lateral geniculate body is distinctly laminated the crossed and uncrossed retinal fibres end in different layers of cells (though always in those parts of the layers which lie immediately adjacent to each other), it is usually not possible to determine the relation of the termination of crossed and uncrossed fibres in more lowly types of mammal, for in these the method of study by transneuronal degeneration is not sufficiently precise to allow of its application. It is known however that in the rabbit (Brouwer and Zeeman, 17), rat (Lashley, 51) and opossum (Bodian, 13) the crossed and uncrossed fibres from corresponding points in the two retinae always end in the same region of the lateral geniculate body (though whether in connexion with the same or immediately adjacent cells is not known). However, a recent experimental study of the geniculate body in the phalanger (Packer, 70) has produced rather surprising evidence that in this marsupial crossed and uncrossed fibres end in relation to alternating laminae of cells, though the laminae are by no means as distinct as they are in the higher eutherian mammals.

The mode of termination of optic fibres in the lateral geniculate body. Earlier studies of Kölliker (48), Tello (80) and Cajal (19), based on Golgi preparations of fetal and immature material, had shown that in the mouse or cat each optic fibre breaks up into a complicated brush of fine terminals enclosing a nest of cells estimated at about 6–8 in number. More recent Golgi studies by O'Leary (66) on the cat have confirmed and considerably extended these observations. He found that the thick axones of the optic tract divide close to the main nucleus of the lateral geniculate body into thick and thin branches. Of these the former actually penetrate into the nucleus, and the terminal ramifications of each one are confined to a single cell lamina and the interlaminar margin

in proximity to it. Within each cell lamina the terminal arborizations overlap each other so that each principal cell makes synaptic contacts with the terminals of several different optic tract fibres. O'Leary also describes in the cat short-axone cells which are distributed throughout the laminae (and distinct from the principal cells whose axones proceed to the visual cortex). The axones of the principal cells may give off a few slender collaterals in the layer to which the cell belongs, but this appears to be "the exception rather than the rule." This last observation, it may be noted, is perhaps in some contradistinction to the previous observations of Tello, who specifically calls attention to the presence of such collaterals. O'Leary, however, believes that his histological evidence is in accordance with the electro-physiological observations that self-reactivation is not an important phenomenon in the lateral geniculate body of the cat, for these observations have demonstrated that fast conduction occurs through the nucleus with no sequelae of sustained activity. He believes, moreover, that the short-axone cells cannot play the rôle of interneurones in the lateral geniculate body, but that they may act as synchronizers for groups of principal cells. An experimental study by Glees (38) of the cat's geniculate body, with the application of refined silver impregnation methods, has demonstrated that, in the mature animal at least, the optic fibres end in the nucleus in special end-formations in the shape of fine terminal rings (entirely similar to the terminal boutons which have been demonstrated in relation to the motor cells of the spinal cord and elsewhere). Moreover, these boutons, as well as the terminal branching of the optic fibres, undergo characteristic degenerative changes after section of the optic nerve. It was estimated that the number of synaptic contacts of optic terminals established with one principal cell amounts to about forty and these contacts are related to the dendrites as well as the cell body. The axo-dendritic contacts, however, are more numerous than the axo-somatic. Lastly, this histological evidence suggested that each optic fibre covers with its terminal branches an area containing about ten cells, and that there is probably an extensive overlap. Similar experimental studies of the geniculate body of the macaque monkey (with the use of the same silver technique) have revealed a rather different state of affairs (Glees and Clark, 39). Here terminal boutons are also present, but in much fewer numbers. They undergo characteristic degenerative changes seven days after section of the optic nerve, and this phenomenon provided the first *direct* evidence that crossed and uncrossed retinal fibres end in different cell-laminae. Each main optic fibre commonly terminates in a spray of 5 to 6 branches (at least in the small-celled laminae). The terminal boutons are in contact with the cell bodies and not with the dendrites, and it was in no case possible to find more than one bouton related to one geniculate cell. It appears, indeed, that in the small-celled laminae of the geniculate body of the monkey each optic fibre is terminally related to 5–6 cells and there is apparently no overlap of the terminals of different fibres. Clearly, such an arrangement would provide for the utmost precision in the recording at the geniculate level of a retinal image. On the other hand, the profusion of synaptic contacts in the geniculate body of the cat, together with the

overlap of different optic fibres, would presumably provide an anatomical basis for a high degree of sensitivity even in low intensities of illumination.

The efferent connexions of the lateral geniculate body. In spite of many statements to the contrary, there is now reason to believe that, at least in higher mammals, the lateral geniculate body projects entirely on to the visual cortex. Thus it has been established by several observers (especially Poliak, 74) that in the monkey all the neurones of the large- and small-celled elements undergo rapid retrograde atrophy following destruction of the area striata of the same side, and this appears also to be the case in the chimpanzee (Poliak and Hayashi, 76). It appears that the geniculate cells similarly undergo total atrophy in the rat (Lashley, 52). According to Bodian (12) occasional residual neurones do persist in areas of degeneration in the geniculate body of the opossum, and this confirms previous observations of Minkowski (63) on the cat's brain. It has already been noted that O'Leary has reported the presence of short-axone cells in the cat's geniculate body. On the other hand, the evidence of retrograde atrophy seems to exclude consideration of such elements in the geniculate laminae of monkeys and apes. Reference may here be made to the contention of Balado and Franke (4) that the neurones of the large-celled laminae in the primates have no connexion with the cortex, but send their axones to the superior colliculus. However, this conception appears to be based on atrophic changes reported to have occurred in the large-celled laminae of the human geniculate body following the destruction of the colliculi by a pineal tumour, and it is doubtful whether this evidence is adequate to controvert the results of careful experiments on lower primates.

The total cell atrophy in the geniculate body which follows ablation of the ipsilateral visual cortex in primates finally demonstrates the absence of any crossed connexions from the nucleus by way of the corpus callosum to the visual cortex of the opposite hemisphere, a connexion which had been postulated by some clinicians to explain the frequent persistence of macular vision after lesions involving the area striata of one side. A similar conclusion follows from Poliak's observation (72) that lesions of the visual cortex never lead to retrograde atrophy affecting the cells of the contralateral geniculate body. This is what might be expected from a consideration of the general organization of sensory systems in the brain for it appears certain that any crossing of ascending sensory impulses which does occur always takes place at infra-thalamic levels.

The absence of any efferent connexions of the geniculate body other than with the visual cortex (such as the geniculo-tectal, geniculo-pulvinar, geniculo-pretectal or geniculo-thalamic tracts which have from time to time been described by comparative anatomists on the basis of the study of normal material) has recently also been affirmed by the experiments of Barris *et al.* (7). These authors produced small lesions in the lateral geniculate body of cats after the corresponding optic tract had undergone complete degeneration. In these cases the ensuing Marchi degeneration was found to be confined entirely to the geniculo-cortical connexions (the optic radiation).

The relation of the lateral geniculate body to the visual cortex. As already mentioned, the experimental evidence leads to the conclusion that in the primates all the cells of both the small- and large-celled laminae project on to the area striata of the occipital cortex. In the macaque monkey, the number of cells in the geniculate body has been computed to be of the order of 1,800,000, and it has further been estimated that approximately 1350 of these cells send their axones to each square millimetre of visual cortex (Clark, 28). Indirect evidence also indicates that the geniculate axones are distributed evenly over the visual cortex. It is interesting to compare these results with data provided by Lashley (54) for the rat. According to these data, there are approximately 34,000 cells in the geniculate body of this animal, while the striate cortex of one hemisphere has a surface area of about 20 sq. mm. Thus in the rat about 1700 geniculate cells project on to each square millimetre of striate cortex.

The axones of the geniculate cells are responsible for forming the fibre laminae characteristic of the lateral geniculate body in the primate brain. Leaving the nucleus, they turn back in the occipital lobe of the cerebral hemisphere, as the optic radiation, to reach the striate area of the cortex. Throughout their course, the fibres of the optic radiation, running in parallel order, remain segregated in bundles in accordance with the site of their origin from the lateral geniculate body (Poliak, 73). It thus appears that the topographical localization of different parts of the retina in this primary optic centre is faithfully carried on through the whole extent of the optic radiation to the visual cortex itself. It may also be noted that it is now entirely agreed that the lateral geniculate body is the exclusive source of the fibres of the optic radiations, and conversely that the lateral geniculate body projects on to no cortical area other than the visual cortex.

Besides the ascending connexions between the lateral geniculate body and the visual cortex, descending connexions have also been described. It is commonly accepted that such connexions form part of a general cortico-thalamic system of fibres whereby all the main nuclei of the thalamus receive corticofugal fibres from the particular areas of cortex on to which they project. It is further assumed that these descending fibres either exert an inhibitory or "damping-down" influence on the sensory activities of the lower centres, or else that they perhaps activate these centres and heighten their sensitivity to in-coming impulses and thus constitute a mechanism of sensory attention (Walker, 84). However, there remains some doubt whether cortico-geniculate fibres actually do exist. They have been described by Biemond (9) in the rabbit on the basis of Marchi preparations, and Mettler (62) with the same technique has affirmed their presence in the monkey. On the other hand, Poliak (73) doubts their existence in the monkey, and Barris (5) was quite unable to detect them in the cat. Clearly this is a matter which demands further investigation with a technique which is more critical than the Marchi method.

The visual cortex. Before considering in further detail the structural basis for the cortical aspects of visual functions, it is desirable to make reference to some of the main features of the visual cortex. In the higher primates, this

area is characterized by the fact that the internal granular layer (Brodmann's lamina IV) is powerfully developed. Moreover, it is split into two strata of cells (laminae IVa and IVc), separated by a fibre layer (lamina IVb). Of the two granule cell layers, the deeper (IVc) is by far the more conspicuous, and there is some difficulty from the morphological point of view in distinguishing lamina IVa from the superjacent pyramidal cell layer (lamina III). The intricate plexus of fibres which is found in lamina IVb forms in the fresh brain a white band usually visible on macroscopic inspection and termed the "stria of Gennari." Hence the visual cortex is often called the *area striata*. In the infragranular layers of the visual cortex (and also to some extent among the fibres in lamina IVb) are occasional large cells, sometimes termed the "solitary cells of Meynert."

In lower mammals, the histological structure of the visual cortex is usually sufficiently distinctive to permit of its demarcation from adjacent cortical areas. In man and other primates, the structure is highly differentiated, so that the transition from the striate area to neighbouring areas is quite abrupt. Hence it is possible to map out the surface extent of the visual cortex with the greatest precision.

Recent studies have confirmed the earlier inferences that the area striata represents the whole of the visuo-receptive cortex. For one thing, Marchi experiments have shown quite clearly that (as already mentioned) the fibres of the optic radiations are distributed to the whole of the area, and to no other area. For another, studies of the electrical activity have demonstrated (in the rabbit) a close correspondence between the optically excitable cortex and the anatomically defined area striata (O'Leary and Bishop, 68). Lastly, the extensive studies of Lashley have demonstrated that, in the rat, the visual functions of the cortex are topographically related to the equivalent cortical area.

The organization of the projection system between the geniculate body and the striate cortex has been determined experimentally by the study of the retrograde cell atrophy which follows discrete and localized cortical lesions. Poliak (74) has found that the smallest cortical lesion (about 1 sq. mm.) which leads to detectable changes in the lateral geniculate body of the monkey always results in the atrophy of a small group of cells in adjacent parts of all six layers of the nucleus. In other words it appears that the projection unit of the lateral geniculate body in respect of the cortex is a band of cells extending radially from the hilum and involving all the cell laminae. Hence, if (as seems certain) the impulses from corresponding points in the two retinae remain distinct within the limits of the geniculate body, they must be brought into the most intimate relation as soon as they are projected on to the cortex, and it may be inferred that the cortical mechanism for the fusion of two images necessary for stereoscopic vision is very localized spatially. Further the three impulses proceeding from each spot in the retina and terminating respectively in the three corresponding laminae of the geniculate body (whatever their functional significance may be) are likewise projected on to the identical spots in the visual cortex where they can undergo immediate synthesis.

The precise site of termination of the fibres of the optic radiation in respect of the various layers of the visual cortex is still in doubt. It has for many years been commonly accepted that the stria of Gennari is mainly composed of the terminal arborizations of these fibres, for atrophy of the stria had been reported in cases of long-standing blindness. Experimental observations on monkeys, however, have not supported this view. For example, the stria remains apparently unchanged in width and density in discrete areas of the visual cortex which had been isolated sufficiently long to allow complete degeneration of all in-coming exogenous fibres (Clark and Sunderland, 32). Rundles and Papez (77) have also reported that in a mangabey monkey and a baboon, in which the optic radiations had been sectioned, the stria showed no evident change in the affected part of the area striata. While, therefore, it is certain that optic fibres penetrate into the stria and enter into its composition, it is predominantly made up of fibre plexuses derived from other sources—probably intracortical connexions of an entirely local character.

The purely histological studies of Cajal had led him to infer that the optic fibres terminate in the visual cortex mainly by arborizations among the granule cells of lamina IV, though he described some extremely fine collaterals which are given off into the infragranular layers. Such collaterals have also been recorded in the cat by O'Leary (67). In the rabbit, O'Leary and Bishop (68) found that the terminal fibres appear to end mainly in the internal granular layer and perhaps also in the deeper part of the lamina pyramidalis. In the cat, they are more nearly confined in the terminal distribution to lamina IV (O'Leary, 67). In higher mammals where the layers of the visual cortex become much more sharply differentiated, it may be supposed that the laminar distribution of optic terminals is correspondingly more sharply defined, and there is some evidence that this is the case. Poliak, on the basis of Marchi experiments in the monkey, concluded that the fibres end almost entirely in lamina IVc though some also reach laminae IVa and IVb. Lastly, the study of degenerative changes affecting the visual cortex after interruption of the optic radiations led Rundles and Papez to infer that the latter terminate, to a large extent if not entirely, in lamina IVc. Poliak's observations seem to show that the optic fibres, consisting of comparatively thick axones, ascend very obliquely into the cortex from the subjacent white matter, and are thus rather distinct from the radiated bundles of fibres which are so conspicuous in the area striata and which appear to be mainly association fibres. This conclusion was strongly substantiated by the study (referred to above) of structural changes in isolated areas of visual cortex in the monkey. In a recent study by O'Leary (67) it is stated that, in the visual cortex of the cat, optic fibres may give off horizontal branches which extend for a considerable distance from the parent stem. "Such observations" (he says) "have significance in that they suggest that a generous area is the minimum size of a cortical point in the usual concept of 'point-to-point' localization." However, he emphasizes that this observation relates only to the cat, and there is reason to suppose that in the primates the distributional area of the optic terminals in the visual cortex must be ex-

tremely limited. Mention should be made of the hypothesis put forward by some authors that crossed and uncrossed retinal impulses are carried separately to each of the two layers of granule cells which comprise lamina IV in the visual cortex of primates. There is, however, no objective evidence for such an assumption.

Association fibres of the visual cortex. Consideration may now be given to the method of diffusion of visual impulses from the point where they impinge upon the cortex. It has generally been assumed that a rich system of association fibres connects the area striata with adjacent and distant cortical areas in the parietal and temporal lobes, and even extends as far forward as the motor area of the precentral gyrus, or toward the frontal pole of the hemisphere. These tracts have been supposed to be the anatomical basis for visuo-tactile and visuo-auditory associations and to provide an essential mechanism for the more elaborate integrative processes related to vision. Surrounding the visual cortex proper are zones of cortex (to which the terms *peristriate* and *parastriate* have been given) which were presumed to be "visual association areas," and it is fair to say that many neurologists held the conception that, while the striate cortex is essentially the receptive area for visual impressions, the latter can be immediately transferred, not only to all parts of the striate cortex itself by intrastriate association paths, but also to the surrounding association areas where visual associations can be formed. However, grave doubt exists in regard to the extent of these association tracts. It seems that their presence has been inferred partly on the basis of gross dissections of the white matter of the cerebral hemisphere (a method which has been shown to be both fallacious and misleading), and partly on the basis of uncritical studies of Marchi reactions following lesions of the visual cortex.

In a recent study (Clark, 27), extremely localized lesions of the visual cortex (in the monkey), carried out with the minimal damage to subcortical structures by the method of devascularization, failed to show Marchi degeneration extending in the area striata itself for more than quite short distances, a matter of 5 mm. at a maximum. In one experiment, degenerating fibres were traced to a narrow strip of peristriate cortex (area 18) immediately adjacent to the striate cortex. Hence it was inferred that an extensive intrastriate association system of fibres probably does not exist, and that association fibres proceeding outside the limits of the striate cortex probably only extend so far as the contiguous peristriate zone. These conclusions are in accordance with recent electrophysiological studies of the visual cortex in the monkey. Combining the method of local strychninization with the recording of electrocorticograms, Dusser de Barenne and McCulloch (34) found that, within the limits of the visual cortex, the strychnine spikes are entirely restricted to the area strychninized and its immediate vicinity, extending for a distance of not more than 1 or 2 mm. In later experiments, Bonin, Garol and McCulloch (14) found that, outside the area striata (area 17), disturbances induced by strychnine are propagated only as far as area 18. Further, strychninization within area 18 leads to disturbances which involve large parts of the same area, and are also

propagated to areas 17 and 19 as well as to the so-called "visuo-sensory band" of the cerebral cortex. Lastly, following strychninization of area 19, no disturbances are propagated to any part of the cortex except that immediately adjacent to the strychnine. On the other hand, strychninization of this area leads to the suppression of the electrical activity of the entire cortex. This last observation suggests the highly interesting conclusion that area 19 must project to some subcortical region which is not reached by the rest of the occipital cortex.

The immediately local effects of impulses reaching any point in the visual cortex suggest that there is no question of the activity of the area striata as a whole being a necessary condition for the completion of visual reactions at this level. This conclusion fits in well with the experimental observations of Lashley and his collaborators on the visual reactions of rats. In the first place, Lashley and Frank (55) sought for a solution of the problem whether any regions surrounding the striate cortex are essential for the retention of a visual habit based upon pattern discrimination. The results of extensive lesions involving the surrounding cortical zones led them to the conclusion either that there is no visual association area which is an essential functional element in habits based on the discrimination of simple geometrical figures, or, if there is such an area, its parts are equipotential for the habits. However, they are of the opinion that "it seems quite certain that no association area outside of field 17 is essential for the retention of the visual habit"; for, in a series of cases, all possible transcortical connexions of the striate areas were severed without abolishing the visual reactions. Subsequently, Lashley (54) found that the ability to discriminate visual figures was retained in animals in which an astonishingly large proportion of the area striata had been destroyed on both sides. In one case the lesion was so extensive that only 700 neurones were left intact in the geniculo-striate system of one side, i.e., about 1/100th of the total normal number. Lashley concludes that the simpler integrative processes involved in visual discrimination and learning may be carried out in a normal or nearly normal manner by a minute remnant of the visual cortex. He further notes that the 700 geniculate cells found effective for detail vision would cover, near the fixation point, 1/200th of the visual field, and that this would be included by a visual angle of about 14°.

It has been proved conclusively by Lashley's work that, in adult rats, destruction of the visual cortex (area 17) results in an inability to discriminate patterns. Experiments by Tsang (83) in young animals, however, suggests that this function may be taken over by the vicarious functioning of other areas. For example, he found that in one experiment in which the visual cortex had been destroyed in a 22-day old rat, pattern vision appeared to be still partly possible, and he notes that "this is inexplicable by the neurological data in hand." However, further observations of a similar kind are required to substantiate and elucidate this conclusion.

The projection of the retina on the cerebral cortex. Having determined that the whole of the lateral geniculate body projects on the visual cortex, the question

arises whether this projection conforms to any plan of localization. Here the evidence is quite unequivocal, for it has been shown that small localized lesions of the visual cortex are always followed by small localized zones of retrograde degeneration in the lateral geniculate body. Indeed, the localization is so sharp and precise that it is possible to speak practically of a point-to-point projection of the lateral geniculate body on the cortex, at least in the higher primates (including man). Even in the lowly opossum a localized visual cortical projection is present, though the precision of localization is not as great as in higher mammals (Bodian, 12). In this animal comparatively larger lesions of the cortex are required to produce distinct areas of complete atrophy in the lateral geniculate body; lesions involving less than 1/60th of the total striate area produce no demonstrable degeneration. In the rat, the localization is rather more precise (Lashley, 52).

By determining the projection of the retina on the lateral geniculate body in monkeys from the study of transneuronal degeneration following small retinal lesions, and the projection of the lateral geniculate body on the cortex by retrograde degeneration in the former following small lesions in the latter, it is obviously possible to map out the whole of the visual cortex in terms of different areas of the retina. Such charts have been completed for the monkey, and clinical observations have allowed the construction of similar charts in the human brain. Indeed, it is now possible to state with considerable accuracy the localized spot on the surface of the visual cortex which receives impulses from any localized spot in the retina.

Poliak's work (73, 74) comprises the most complete presentation in recent years of the organization of the projection system in the monkey from the retina through the lateral geniculate body to the striate cortex, and for the details of the retinal representation in the striate cortex of the monkey's brain reference may be made to his monographs. That a similar organization exists in the anthropoid apes has been shown by Poliak and Hayashi (76) for the chimpanzee, and in man by a variety of observations on clinical cases (see especially Brouwer, 16, and Balado and Franke, 4). It may be emphasized that in the area striata of each cerebral hemisphere homonymous halves of both maculae as well as homonymous halves of extramacular quadrants are represented. There is no question, therefore, of a bilateral cortical representation of the entire macula. Poliak has shown that topographical factors as well as the details of vascular supply explain why central vision is so frequently retained in pathological lesions of the occipital lobe in man.

Intrinsic organization of the visual cortex. Much requires to be done in the analysis of the neuronal pattern of the visual cortex in order to determine in what possible directions the diffusion of in-coming visual impulses is likely to occur. The study of Golgi preparations of the visual cortex of primates has given some general indication of this pattern. For example, it is evident that the short-axone cells of the internal granular layer (the functionally receptive layer) can serve as interneurones through which visual impulses may be transmitted *1*, to the apical dendrites of the fusiform cells in laminae V and VI;

2, to the dendrites of cells of Martinotti in lamina V and thence by their ascending axones to the zonal or plexiform layer on the surface of the cortex; *3*, to the basal dendrites of the large pyramidal cells in lamina IVb (whose axones probably proceed to sub-cortical centres), or *4*, to other short-axone cells by relays of which they can be diffused through the horizontally-disposed plexus of the stria of Gennari. The majority of the pyramidal cells of the cortex, including the large solitary cells of Meynert, can be activated through their apical dendrites by impulses which are diffused through the fine fibres of the plexiform or zonal layer. It is further evident that these impulses reach the zonal layer *1*, by the ascending axones of the cells of Martinotti; *2*, by the vertically-running fibres of the radiated bundles, which are predominantly association fibres derived from immediately neighbouring areas of the cortex, and *3*, by the fine ascending collaterals of the main axones of the pyramidal cells. It is to be hoped that such inferences, based on purely histological observations, will in future studies be confirmed and extended by the application of electro-physiological methods. In a recent study of the optically excitable cortex in the rabbit's brain by O'Leary and Bishop, attention has been brought to the possibilities of such a technique, and also to its limitations. With regard to the latter, the authors point out that the axonal substrate of the cortex shows no dominant orientation throughout; in any one plane conduction of axone impulses evidently proceeds in opposite directions at the same time so that the summation of the potential which would result would be algebraic rather than simple; all axones or collaterals which pass one electrode placed in a given dimension of the cortex may not pass another situated a short distance from it; activity in a single axone and its collaterals may progress in opposite directions simultaneously. They further emphasize the complications introduced into the anatomical pattern from the standpoint of electrical recording by the profusion of short-axone cells in the visual cortex. These cells, it is considered, may provide for the formation of reverberating circuits or self-re-exciting chains, and may also act as synchronizing agents. Lastly, O'Leary and Bishop draw particular attention to the widespread origin of the axones and collaterals which contribute to the plexuses of the lamina zonalis, on the basis of which it is inferred that "the activity of the plexiform layer should reflect the status of activity in all subjacent layers of the cortex."

The efferent elements of the visual cortex. If the geniculo-striate organization can provide the anatomical basis for visual reactions independently of surrounding cortical areas, it is clear that there must exist efferent connexions through which the visual cortex is brought directly into relation with motor mechanisms of the brain-stem and spinal cord. There is some reason to believe that these connexions are mainly established by the axones of the large ganglion cells of lamina IVb and of the large solitary cells in lamina VI, for these cells undergo a partial degeneration when the subcortical connexions of the area striata are interrupted, while other cellular elements of the cortex show remarkably little change. On the other hand, it must be admitted that retrograde degeneration is not an altogether reliable index of the extent and distribution of

efferent neurones in the cortex, for the numerous collaterals given off from the proximal part of the axones are apparently capable of permitting their survival even after section of the main distal part of the axone.

Cortico-pontine connexions. Cortico-pontine connexions from the area striata have been both affirmed and denied for a number of years by various authors. In recent years the work of Sunderland (78) has contributed additional evidence for their existence in the monkey. Mettler (62), however, concluded that none are derived from the macular region of the area striata, and he was uncertain whether any come from the extramacular regions. In the cat, cortico-pontine fibres from the striate cortex have been described by Barris, Ingram and Ranson (7), confirming earlier observations made on the same animal by Poliak (72). Probably the question of these connexions will not be finally settled until some better technique can be applied to its elucidation.

Cortico-mesencephalic connexions. The cortical control of the lower visual centres in the mid-brain is an important element in the physiology of vision. It is mediated by descending fibre paths from certain discrete areas of the cortex, though the precise anatomical details of these paths are still not fully known. In a recent review of the subject, Holmes (46) has discussed the cerebral integration of ocular movements, mainly from the clinical point of view. He refers to the well-established fact that ocular movements can be induced by stimulation of an area in the frontal cortex and also of the cortex of the occipital lobe, and he makes the following statements regarding the contrast in the functional significance of these two oculomotor regions—"All evidence at our disposal indicates that the frontal oculomotor centre is concerned in those movements and reactions of the eyes which we may call voluntary. Through it we can by an effort of will look or turn our eyes in any direction and converge them on a near object." On the other hand, "the functions of the occipital centre are more numerous. Fusion, that is, the reactions necessary to unify the separate impressions from the two retinae, and accommodation depend on it. The movement of the eyes to an object outside central vision, in so far as it is involuntary, is also excited through the occipital cortex by extrafoveal impulses. And finally the maintenance of fixation on a point, whether it is at rest or in movement, is determined by a cortical reflex mechanism in the occipital lobes."

It is presumed that the fronto-mesencephalic fibres descend in the medial part of the cerebral peduncle, and, entering thence the tegmental region of the mid-brain, terminate in connexion with the superior colliculus, or more directly with the oculomotor nucleus. The cortico-mesencephalic fibres from the occipital lobe, on the other hand, commonly form quite a well-defined fasciculus which, in Weigert sections, can be seen penetrating the base of the pulvinar, and entering the deeper part of the stratum opticum of the superior colliculus. There is some doubt whether these fibres have their origin in the visual cortex proper (as generally supposed in the past). Poliak (73), for example, believes on the basis of his experiments on monkeys that they are derived rather from the peristriate cortex, and Foerster (35) reported that in

experiments on the human brain he was unable to elicit ocular movements on stimulation of the striate area alone, while they could be obtained on stimulation of the peristriate areas (areas 18 and 19). On the other hand, in a recent paper Walker and Weaver (85) state that they were able to elicit contralateral conjugate deviation of the eyes in monkeys by stimulation of the cortex of the area striata. Moreover, they observed some evidence of localization in this motor projection system, for when electrodes were placed above the calcarine sulcus, the movements tended to be lateral and downward, while if placed below the sulcus, the movements were rather lateral and upward.

It was stated above that in normal Weigert sections the cortico-mesencephalic fibres can be seen to enter the superior colliculus. This does not preclude the possibility that some of them also terminate in deeper parts of the mid-brain. Indeed, there is evidence that this must be so, and we may refer back to the old observations of Bernheimer (8) that movements of the eyes on stimulation of the occipital cortex can still be obtained after destruction of the superior colliculus. More recent anatomical studies have provided further evidence. In the rat (Clark, 26) fibres were traced from the cortex adjacent to the area striata to the pretectal nucleus, and a similar observation was made on the cat's brain by Barris (6). The latter also showed that stimulation of the cortical area in question (which in the cat is quite restricted) gives rise to an equal constriction of the pupils, and thus may be referred to as a specific "pupillo-constrictor area" of the cortex. Lastly, according to Mettler (62), in the monkey, cortico-mesencephalic fibres arise from the striate cortex in the region of the calcarine sulcus (but not from the macular area) and also from the peristriate areas. This author also states that from the upper lip of the calcarine sulcus descending fibres extend to reach the oculomotor nucleus, the trochlear nucleus, the central gray matter of the cerebral aqueduct, and the fasciculus longitudinalis medialis.

It will be observed that not only do the cortico-mesencephalic connexions provide an anatomical basis for the cortical control of ocular movements; they may also convey impulses which can be relayed through the tectum to motor centres of the brain-stem and spinal cord by tecto-bulbar and tecto-spinal tracts.

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TISSUE CHANGES IN VITAMIN DEFICIENCIES

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The vitamins are naturally occurring organic substances which possess unique molecular structures that the organism is unable to synthesize but which are essential components of the chemical machinery of cells. Since most organisms cannot synthesize these particular molecular configurations, such substances must be supplied preformed in the food.

Pathology is the science concerned with the responses of living organisms to injurious conditions. Non-lethal factors in the course of time have led either to patterned reparative processes correlatable with normal growth sequences or to gradual adaptations which have led to species differentiation. The more ancient the conflict between organisms and environment, the more definitely patterned are the responses, notably those following physical injury.

Deficiency of a single vitamin in the nutrition of an organism creates a condition highly improbable of occurrence in nature. Therefore, the study of the consequences of vitamin deficiencies affords a new and novel approach in pathological exploration.

The morphological changes of the vitamin deficiencies reflect the injury caused by defective chemistry of cells. In a given deficiency, this kind of injury may be peculiar to one type of cell or more generally distributed in several tissues dependent upon the particular biochemical process affected and the importance of the process to the cell type. Where possible we should distinguish between primary effects (the immediate consequences of a deficiency upon tissues in which the vitamin is operative) and secondary effects—the effect upon the organism as a whole in consequence of the loss of function of the tissues primarily affected and in general manifested by inanition. Other characteristics of the vitamin deficiencies are: *a*, that great variety in the nature of tissue injury is obtainable because of the numerous deficiencies; *b*, that differences in the degree of the deficiency may lead to a difference in response; *c*, the nature and/or degree of the response may be influenced by the state of biological activity of the organism (growth, other deficiencies, infection, etc.); *d*, that injuries of this type are experimentally reproducible—which offers great advantages for study.

The morphologic effects of a vitamin deficiency, to have scientific value, should be correlatable with known normal sequences, for which purposes the study of processes of repair following replacement therapy are usually of greatest value.

In addition to the value of this method of approach in the study of tissue responses, vitamin deficiency experimentation is a means of chemical micro-dissection of cells and affords possibilities of relating function with structure that have not yet been seriously explored.

Naturally, our selection of material has been chiefly from reports of experimental work and we have included biochemical and physiological data whenever

deemed helpful in elucidation of morphological problems. We have avoided discussion of priority and have consciously excluded numerous papers from our list of references, either because of lack of merit or because their contents were adequately summarized in the publications we have included. It has seemed advisable to incorporate a few of our unpublished observations, particularly those regarding the changes in hypervitaminosis A and on riboflavin and pyridoxine deficiencies.

VITAMIN A. A review by Bessey and Wolbach in 1939 (25) contains an adequate and carefully selected list of references to the physiology of vitamin A and to the pathology in man and animals caused by its deficiency. Other references are to be found in a non-critical review by Robertson (252).

We shall limit ourselves to brief considerations of the consequences of vitamin A deficiency upon: a, epithelial structures; b, the incisor teeth of rodents, and c, the skeleton. While there are marked effects upon the hematopoietic tissues, we have no proof that they are different from the inanition effects of several other vitamin deficiencies.

Epithelial tissue. The changes produced in many epithelial structures are to be regarded as the most characteristic consequence of vitamin A deficiency because they appear regardless of age and presumably in all vertebrates, though demonstrated as yet only in man, monkeys, cattle, swine, dogs, rabbits, guinea pigs, rats, mice and fowls (335). The sequence of the epithelial effect may be epitomized as follows: atrophy of the epithelium concerned, reparative proliferation of basal cells, and growth and differentiation of the new products into a stratified keratinizing epithelium. Regardless of the original function and structure of the region, this replacement epithelium is identical in all locations and comparable in all its layers with epidermis (329) (330) (115) (306) (35).

The order of the response of various organs varies somewhat with different species of animals but essentially the same organs are involved. The keratinizing metaplasia is found in: *a*, salivary glands, including the submaxillary, parotid and all accessory glands of the buccal cavity, tongue, and pharynx; *b*, the respiratory tract, including the nares, maxillary sinuses, Jacobson's organ, trachea and bronchi; *c*, genito-urinary tract, including the renal pelvis, ureters, bladder, epididymis, prostate, seminal vesicles, coagulating glands, uterus, oviducts, and accessory sex glands of the vulva; and *d*, eyes and paraocular glands, including the corneal and palpebral conjunctivae, the Harderian, intra-orbital and extra-orbital, and the Meibomian glands. In man, hyperkeratotic lesions of the skin centering about hair follicles may occur in the deficiency after puberty (109).

The mucosa of the stomach and intestines and the renal tubules do not undergo the above described changes. At most, some degree of atrophy of the intestinal glands may be attributed to the deficiency. Ulcers in the forestomach of the rat following hyperkeratosis which have been attributed to vitamin A deficiency are not peculiar to this condition (224).

In general, in vitamin A deficiency the epitheliums which atrophy and which become replaced by stratified keratinizing epithelium are those which have a secreting (chemical) function in addition to the rôle of a covering layer and whose

functioning cells are without power to divide. Repair, therefore, takes place from focally distributed basal cells which multiply, spread beneath the original epithelium, and finally, through coalescence of areas thus produced, form a continuous epidermis-like layer. On the other hand, epithelial cells with chemical rôles, as liver and renal tubules, which do have the power of dividing, do not exhibit marked degrees of atrophy nor are they replaced by keratinizing epithelium. Certain stratified epitheliums—cornea, renal pelves, ureters, and bladder—in vitamin A deficiency increase their growth rate and become hyperkeratotic, which might be interpreted as evidence that these epitheliums normally have specialized functions which are inhibited by the deficiency. No satisfactory explanation has been found for the fact that the reparative activities of basal cells of many different epitheliums in vitamin A deficiency end in an identical stratified keratinizing epithelial product. A natural assumption is that vitamin A is necessary in some chemical process uniquely related to normal differentiation and life of this type of cell.

In recovery following vitamin A administration, in spite of the common morphology, the epithelium of each region returns to its normal type in morphology and function. The recovery sequences have been followed by Wolbach and Howe (331) and some of their conclusions are: "all cells of the lowermost layer of the replacement epithelium have proliferative powers as in the stratum germinativum of epidermis"; "The important histological features of repair involve removal of the layers of cells irreversibly differentiated toward keratinization and direct differentiation of the stratum germinativum into the normal type. These take place simultaneously."; "The histological sequences observed in the removal of cells above the stratum germinativum indicate that autolysis as shown by vacuolar degeneration and heterolysis as shown by leucocytic infiltration are involved."

In repair the vacuolar degeneration separates the replacement epithelium into two strata, the upper to disappear completely, the lower to reproduce the epithelium normal for the region. The sequences are very similar to those in the rat's vagina in the di-estrus. As the metaplasia of vitamin A deficiency and its recovery is a cycle that probably does not occur in animals in natural habitats, it is of great interest to note that the recovery phenomena follow a familiar physiological pattern.

Perhaps the fact of greatest interest in vitamin A deficiency is the preservation by the cells of the stratum germinativum of the replacement epithelium, of the identity of the original epithelium throughout the period of metaplasia. Wolbach and Howe (331) found that the cells of this layer can assume the original morphology and functions without undergoing division when supplied with vitamin A and inferred that the nuclear chromatin remained unaffected by the deficiency. In the opinion of these authors, the cycle of vitamin A deficiency and recovery affords an experimental method available for the correlation of nuclear chromatin and types of cytoplasmic activities. Searching cytological studies by competent persons would probably bring to light new relations of structural detail and physiological activities.

Mellanby, who at one time (204) (205) suggested that the epithelial changes of vitamin A deficiency are secondary to lesions of afferent nerves, has very recently (207) suggested, in disregard of the actual sequence of events, that the "fundamental change is overgrowth of epithelial cells of all kinds, keratinization and metaplasia being secondary to this overgrowth."

Teeth. Vitamin A deficiency produces profound changes in the incisor teeth of rats and guinea pigs because these structures grow continuously throughout the life of the animal and because the leading rôle in the organization of the tooth at the formative end is played by the enamel organ, an epithelial organ which in vitamin A deficiency atrophies and undergoes keratinizing metaplasia. Following the enamel organ atrophy, there is atrophy and failure of polar deposition of dentin matrix (predentin) on the part of the odontoblasts—cells of mesenchymal origin. The odontoblasts on the labial side of the tooth remain normal in appearance and continue to deposit dentin matrix in apposition to the enamel organ long after complete disappearance from the other sides.

After complete enamel organ atrophy in the rat, the odontoblasts disappear also on the labial side. Before they completely lose their identity, they lose their columnar shape but continue to deposit dentin matrix, but on all sides—in centrifugal fashion, like osteoblasts. Wolbach and Howe (332) whose observation we have been quoting, characterized the odontoblast as a polarized osteoblast and regard the enamel organ as the polarizing agent. They also described, in complete vitamin A deficiency, total failure of dentin formation and inclusions of enamel epithelium brought about by plication, occasioned by stress upon imperfect dentin. The above processes bring about a marked change in the structure of the tooth, abnormally thick dentin on the labial side and abnormally thin elsewhere. In recovery following vitamin A administration, enamel organ regeneration takes place and new formation of dentin is resumed by cells, apparently derived from the pulp, before they have assumed the normal columnar shape of odontoblasts.

Boyle (41) has described, in the tooth germs of a human infant with vitamin A deficiency, changes in the enamel organ comparable to those in rodent incisor teeth.

The work of Wolbach and Howe is confirmed, with but minor reservations, in a thesis in 1938 by Pohto (240). Schour, Hoffman and Smith, in a recent publication (262) also confirm on the whole and extend the findings of Wolbach and Howe. Their paper is valuable for references, for an admirable account of the histophysiology of incisor teeth of rats, additional observations and exact nomenclature. They point out that "the reaction in vitamin A deficiency offers ideal material for the analysis of a number of physiologic processes in tooth development. Their conclusions of greatest significance agree in attributing organizing influence of the enamel organ (odontogenic epithelium) upon the incisor tooth throughout the life of the animal, and they call attention to differences in the rôles of the lingual and labial odontogenic epithelium. The dentin covered by enamel (labial side) they show, forms at an accelerated rate, while the cementum-covered dentin (lingual dentin) forms at a decelerated rate. Orten,

Burn and Smith in 1937 (222) (52) by subjecting rats to long periods of incomplete intermittent vitamin A deficiency, obtained tumor-like formations and tooth duplications at the formative end of the incisor teeth. This is to be explained by the organizing action upon mesenchymal tissues of portions of the odontogenic epithelium which became displaced or isolated as the result of mechanical effects upon the defective dentin near the formative end.

The reviewers believe that careful reading of the papers cited on teeth (332) (41) (240) (262) (52) will suggest a number of problems we have not mentioned which are amenable to analysis by means of vitamin A deficiency and repair experiments.

Bone and nervous system. Paralysis and nerve degeneration as a consequence of vitamin A deficiency have been affirmed and denied by a number of investigators (25) (337). It has been known for many years that vitamin A deficiency retards growth of bone and in particular endochondral bone formation (329) (305) (146). This effect has not been regarded as different from that of inanition, however produced. Recently it has been shown by us (336) (337) that if vitamin A deficiency is established at a sufficiently early age, skeletal growth becomes retarded in a unique manner a considerable period before the rate of increase in weight is materially affected. The central nervous system and other soft tissue continue to grow at approximately their normal rate until general inanition effects appear, as shown by stationary weight or loss of weight.

The effects of this disproportionate growth of bone and central nervous system are: *a*, overcrowding of the cranial cavity, resulting in distortion of the brain, dislocation toward the foramen magnum and multiple herniations of the cerebrum and cerebellum into the venous sinuses of the dura at sites of arachnoidal villi; *b*, overcrowding of the spinal cord and herniations of nerve roots into intervertebral foramina and into the bodies of vertebrae; *c*, mechanical damage with subsequent irregular degeneration of the nerve roots, peripheral nerves and of nerve fibers in various tracts of the spinal cord and in the brain.

The reparative powers of the neurons are not impaired—at least before the effects of general inanition become apparent—as evidenced by the prompt appearance of axon regeneration phenomena on the proximal side of the mechanically damaged nerve roots adjacent to herniations.

The nervous lesions of vitamin A deficiency thus are wholly mechanical in origin.

Weanling rats (21 days old) on a vitamin A deficient diet usually exhibit signs of nerve lesions by the 54th day of age. The later the deficiency becomes established, as shown by the effect upon growth, the less frequently does the disproportionate growth reach a degree sufficient to produce compression of the central nervous system. Vitamin A administered at 42 days of age will prevent paralysis, although dissection may show slight degrees of relative overgrowth of the nervous system. If the vitamin deficient rat is prevented from growing by an insufficient amount of food, the nervous system preserves relatively normal relations to the skeleton and no paralysis ensues. Rate of growth and not age is the important factor in the production of the disproportionate relations. Rats

maintained at a small size by insufficient amounts of a complete diet into an age period in which, in normally nurtured rats, it is not possible to produce paralysis, exhibit the disporportionate growth just as rapidly as do weanlings when placed upon a vitamin A deficient diet.

We have found a similar disproportionate growth of bone and central nervous system in young vitamin A deficient guinea pigs.

The prompt retardation of endochondral bone formation in vitamin A deficiency indicates that a specific action must be involved, but no distinctive histological appearance has been found. Osteogenesis *per se* is not inhibited because bone matrix (osteoid) formation continues late into the deficiency. We have confirmed the formation of excess periosteal bone in relation to the bony labyrinth of the ear in dogs, as reported by Mellanby (206) and have recorded (337) similar findings in rats and guinea pigs. We have not found excess bone formation in rats in other parts of the skeleton. There is no explanation for the bone formation of periosteal origin in relation to the bony labyrinth of the ear. One premise of promise is that the bony capsule of the labyrinth attains adult size before birth. However, meager information at hand (213) (214) indicates that in the calf, bone proliferation in relation to the optic foramina is the conspicuous intracranial productive response of bone to vitamin A deficiency. The solution probably will require accurate information about the order of development of centers of ossification and the progress of endochondral bone formation at the base of the skull. Also, the effect of pressure upon the cranial bones, in general, must be analyzed.

Mellanby in a recent paper (207) describes in dogs overgrowth of bones; of the cranial bones, particularly those forming the posterior fossa, the vertebrae and femurs. He regards this overgrowth as related to degenerative changes in the brain, cranial and peripheral nerves. He states that "A function of vitamin A is to influence the structure of growing bone, probably by limiting the number and degree of activity of osteoblasts and osteoclasts. In its absence from the growing dog, osteoblastic and osteoclastic activity is increased, thus resulting in proliferation of cancellous at the expense of compact bone and causing many bones to lose their normally fine moulding and outline and to become thickened and enlarged." We cannot find, in vitamin A deficient rats, premises corresponding to Mellanby's in regard to osteoblastic and osteoclastic activities. We do not understand his statement in explanation of bone overgrowth.

Investigation of the consequences of vitamin A deficiency upon growth before weaning and in late prenatal periods promises to be informative. It is possible that the rosette formations of the retina in very young rats, described by Johnson (157), may be the result of more rapid growth of the retina as compared with that of the eyeball. A short paper by Hale (132) reports anophthalmos and other arrests of development in pigs born by vitamin A deficient sows. Mason (189) has investigated the effects of vitamin A deficiency in the pregnant rat. Fetal death is produced as a result of placental degeneration in contrast to the vitamin E deficiency effect which is upon fetal tissues. No significant injury of ova or impairment of implantation was found. Mason could find no evidence

of disturbances of endocrine functions of ovary and anterior hypophysis. Another bit of evidence that the epithelial changes in vitamin A deficiency are unrelated to endocrine physiology was produced by Mason and Wolfe (190) who found that castration (males and females) was without effect upon epithelial responses.

Atrophy of the testis in vitamin A deficiency has been known for many years (335) (187) (255). In our opinion, the atrophy of the seminiferous tubules in vitamin A deficiency as in other epithelial organs spares the undifferentiated cells, and hence recovery is possible with replacement therapy.

The consequences of vitamin A deficiency upon hematopoiesis have not revealed specific features. There is, however, in comparison with the anemias associated with other deficiencies, a heavy deposition of hemosiderin in the liver and particularly in the spleen. In replacement therapy recovery, following an outburst of erythroblastic activity in bone marrow and spleen, the hemosiderin rapidly disappears from the organs, which is presumptive evidence that the iron stored in phagocytic cells is utilized (334).

Hypervitaminosis A. Conflicting opinions are expressed (92) (310) (62) regarding the consequence of the administration of excessive amounts of vitamin A. The effects first described by Collazo and associates (63) (64) (65) of inanition, exophthalmos, loss of hair, and multiple fractures of the long bones produced by excessive doses of fish oil concentrates have been confirmed by a number of workers (289) (319). No confirmatory experiments by the use of pure vitamin A have been published, and naturally other substances in the concentrates have been suspected of producing the untoward consequences. No adequate pathological study has been reported of the sequences in skin and bone. The early bibliography of this subject is given by Wahlin (315) who reviews Agduhr's many pioneer publications on the deleterious effects of excessive amounts of cod liver oil.

Strauss (289) reported degenerative changes in kidney, liver, spleen and heart muscle, and atrophy of seminiferous tubules. The bones showed retardation of osteogenesis, cessation of endochondral bone formation, but no general resorption of bone. Osteoclasis was marked only in regions of fractures. Other than this brief and wholly inadequate account, we could find no microscopic descriptions recorded for the bone lesions produced by fish oil concentrates of vitamin A.

Vedder and Rosenberg (310) believe that the fish liver oil concentrates contain an unidentified toxic substance which is responsible for the lesions attributed to vitamin A. They found that vitamin D in proper dosage partially counteracts the toxicity for rats of the jewfish liver oil they used. Ascorbic acid, they found, almost completely counteracted the toxicity of jewfish liver oil for rats.

A few unpublished experiments by us have shown that the major effects reported by Collazo et al. are produced in young rats in 7 to 11 days by the daily administration of pure vitamin A[1] in amounts of 30,000 to 40,000 international units.

[1]Crystalline product obtained from the Distillation Products, Inc., Rochester, New York.

No important change has been seen by us in any of the internal organs. Heart, lungs, liver, kidneys, pancreas and gastrointestinal tracts were essentially normal. The adrenals showed loss of lipoid vacuolization and some atrophy of the glomerular zone. The spleen in some instances was enlarged and actively hematopoietic. Skin lesions of eyelids and adjacent regions and snout had a considerable resemblance to vitamin B_6 (pyridoxine) deficiency lesions. The effects upon bone were dramatic. We found that 24 hours' fixation in Zenker's fluid was sufficient to decalcify, for sectioning purposes, the vertebral column and the shafts of the long bones, indicating marked decalcification. We are not prepared to give an adequate description of the processes, which are very apparent in the bones, nor to interpret what we have seen in terms of familiar sequences. The conditions leading to fractures evidently are decalcification and osteoporosis, accompanied by large numbers of osteoclasts. Osteoporosis was most marked in regions where remodelling of bone is a normal growth process, such as the ends of long bones and at the curvature of the tibia. The sites of fractures seemed to be determined in part by the degree of bone resorption and to the resultant of forces of muscular action.

Early effects of the hypervitaminosis in bone are: *a*, the presence of increased numbers of fusiform and osteoblastic cells in the periosteum; *b*, increased osteoclasis near the epiphyseal ends of bone and other regions where remodelling is normal to growth; *c*, small hemorrhages between bone and peirosteum resembling early scorbutic lesions; *d*, apparent acceleration of growth and cytomorphosis of cartilage in endochondral bone formation; *e*, abundant osteoid in endochondral bone formation. Reparative processes in callus formation are reminiscent of those in experimental scurvy, but we have as yet no accurate knowledge of the duration of the fractures we have studied. Organization of hemorrhages into the tissues seems delayed. Osteoblasts are present in great numbers but osteoid deposition seems reduced in amount or delayed. In vertebrae and sternum, resorption of bone with accompanying osteoclasis is very striking, while endochondral bone growth processes in contrast to that in the long bones are more or less retarded.

Examination of the teeth has shown little of histological importance. Very striking bone resorption without typical reparative process was found in the adjacent supporting bone in regions most subject to stress.

Study of a larger series of animals with particular reference to histological sequences and to reparative sequences after withdrawal of vitamin A excess may require modification of some of the above statements; nevertheless, the destructive action upon bone of vitamin A as represented by the above dosages is a matter of fact. The histological evidence points to the organic matrix as the seat of the important or perhaps the initial disturbances which culminate in fractures. A hypothesis we hold at the present time is that the early consequence of excessive vitamin A administration is the acceleration of some processes of bone growth, notably: *a*, periosteal proliferation; *b*, epiphyseal cartilage sequences preliminary to endochondral bone formation, and *c*, remodelling of bone attended by osteoclasis. The vitamin A intake in these experiments was far in excess of that likely to be administered to humans.

Cornil et al. (66) in the guinea pig describe as visceral effects of hypervitaminosis A, focal necroses of the liver, enlargement of the islands of Langerhans, congestion of the spleen with ochre colored intracellular and extracellular pigmentation (hemosiderin?), hyperactive spermatogenesis and hyperkeratinization and desquamation of the skin. In the pituitary (67) in severe hypervitaminosis A, they describe loss of acidophilic cells and in vitamin A deficiency, the presence of pseudo-adenomatous accumulations of acidophilic cells.

It is not possible to appraise these two short papers on the basis of our own work with rats.

VITAMIN C DEFICIENCY—*Ascorbic acid.* The morphologic consequences of experimental ascorbic acid deficiency are practically restricted to supporting tissues of mesenchymal origin and may be expressed by the statement that there is failure of formation and maintenance of intercellular materials (328) (333). This definite and simple characterization explains the pathology of experimental ascorbic acid deficiency in susceptible animals and in the naturally-occurring deficiency in man (scurvy). We therefore do not think it important to describe in detail the lesions of human ascorbic acid deficiency (scurvy) or much of the early experimental work on guinea pigs. Hemorrhages, loosening of teeth, failure of wounds to heal and, in infants, extensive subperiosteal hemorrhages and separation of the epiphyses of long bones are manifestations of scurvy and all are explainable on the above simple pathologic principle.

For the pathology in man, consult Aschoff and Koch's monograph (13), the monograph of Park and his associates (232) and for human tooth pathology, Westin's monograph (320). These monographs and the book by Hess (145) give access to the important literature. An early important monograph dealing with experimental scurvy in guinea pigs is that of Höjer in 1924 (149).

The guinea pig has been used almost exclusively for ascorbic acid deficiency experimentation. In young guinea pigs on a diet completely deficient in ascorbic acid, microscopic evidence of retarded deposition of intercellular matrices, bone, connective tissue and dentin can be found as early as the seventh or eighth day (328) (46) (73). Finally, all deposition of intercellular material in growing structures ceases and there is slow resorption of the matrices previously laid down (328) (74) (111).

That the outstanding effect in the production of the pathology of ascorbic acid deficiency is failure in the formation of intercellular materials is generally accepted. Recently it has been verified in the healing of wounds in experimental human ascorbic acid deficiency (71) (153).

Wolbach and Howe (328) (333) and Hunt (153) believe that ascorbic acid is not necessary for long survival and multiplication of the cells concerned with matrix formation. The deficiency is specifically concerned with the elaboration of intercellular substance, which they regard as the product of cells. They describe in some detail (328) the change in morphology of osteoblasts that accompanies the failure in bone matrix production. In the incisor teeth of guinea pigs, the columnar odontoblasts undergo changes in size and shape which Höjer (149) and Fish and Harris (106) regard as degenerative but which in our opinion represent a change in morphology accompanying loss of a function. In

each instance, the bone and dentin forming cells take on the appearances of undifferentiated connective tissue cells.

All intercellular substances of the supporting tissues, bone, cartilage, fibrous connective tissue and dentin, have a common substructure of collagen. By collagen we wish to indicate a number of compounds of similar though not necessarily identical composition which react similarly to staining techniques. It is this protein substructure which in scurvy is either not produced or is produced in defective form.

Wolbach and Howe interpreted appearances in the incisor teeth of guinea pigs and in bone (gerüstmark) as indicative of the presence of a liquid. The prompt appearance of dentin and bone matrices in considerable volume following ascorbic acid administration, led them to advance the theory that the failure of cells to produce an intercellular matrix in scorbutus is the result of the absence of an agent common to all supporting tissues which is responsible for the setting, fibrillation, or jelling of a product which would otherwise remain liquid.

Some of their observations in regard to tooth changes were faulty, as has been pointed out by Ham and Elliott (138) and MacLean, Sheppard and McHenry (181). The results of the studies of Boyle, Bessey and Howe (46) are also not in accord with the interpretations of Wolbach and Howe (328) whose errors were in part the result of artefacts, in part the result of their failure to demonstrate amorphous calcified material between the layer of odontoblasts and normal dentin because they used Zenker's fixative. The disappearance of this calcareous material gave appearance as if an empty zone existed, an appearance which, however, is indicative of the presence, at least, of an exceedingly tenuous matrix. Neither Ham and Elliott (138) nor MacLean and associates (181) employed the recovery type of experiment in their studies. The latter, without adequate reasons, asserted that the deposits of dentin in the repair experiments of Wolbach and Howe were the effects of the deficiency before therapy. (Glasunow's (111) description of new formation of dentin in guinea pigs in recovery from scorbutus is like that of Wolbach and Howe's.) The former did not study animals in complete ascorbic acid deficiency. Neither group, because of the character of their material, was qualified to pass judgement on the "jellation theory." Dalldorf (74) (92), on the basis of his own experiences, adheres to the "jellation theory." Details brought to light in the study of the formation of collagen in recovery from experimental scorbutus (333) may be interpreted as supporting the "jellation theory."

Mazoue (198) studied sequences in granulation tissue induced in the peritoneal cavities of guinea pigs in scorbutus and in recovery following ascorbic acid therapy. He regarded his observations as confirming the work of Wolbach and Howe, though he made no specific mention of the "jellation theory."

Probably the most careful study of dentin formation is that of Boyle, Bessey and Howe (46). By the use of spaced alizarin injections they followed the rate of dentin formation in the incisor teeth of guinea pigs while on diets containing restricted amounts of ascorbic acid. They demonstrated a measurable quantitative relation between the rates of formation of dentin and the amount of

ascorbic acid administered. In guinea pigs receiving 0.1 mgm. of ascorbic acid daily, as well as those on an ascorbic acid free diet, the normally uncalcified predentin was absent. This paper contains proof that the mechanism of calcification is not affected in ascorbic acid deficiency, confirming the generally accepted conviction. Elsewhere Boyle and collaborators (43) state that in vitamin C deficiency, dentin deposition stops and previously deposited pre-dentin becomes heavily calcified. Odontoblasts become morphologically indistinguishable from other pulp cells. Boyle's study of enamel formation in guinea pigs in ascorbic acid deficiency (45) contains further proof of the continuance of normal calcification. The enamel organ, unlike mesenchymal sources of calcified structures, is not primarily affected in scorbutus in the guinea pig or in the tooth germ in human infantile scurvy (42). Park and associates (232) showed that in human scurvy, calcification of cartilage trabeculae at epidiaphyseal junctions may be more intense than normally.

The continuously growing incisor teeth of guinea pigs provide perhaps the best opportunity for the study of the effect of ascorbic acid deficiency upon formation of a matrix. The evidence that dentin-matrix (predentin) formation ceases is complete and presumably most workers would agree that as the period of the deficiency extends, dentin formation becomes more and more subnormal—quantitatively and qualitatively. Whether or not the odontoblasts degenerate and disappear or simply lose their morphology and become indistinguishable from other pulp cells must be regarded as unsettled. The fact that the subnormal dentin last deposited becomes heavily calcified is of importance concerning the chemistry of calcification. The microscopic appearance of the remains of this dentin after decalcification indicates an extremely tenuous matrix.

Less work has been devoted to the study of bone matrix (osteoid) formation in ascorbic acid deficiency. There is general agreement that osteoid deposition ceases and that osteoblasts lose their morphology and take on the appearances of fibroblasts (328) (74) (106) (181) (111). Fish and Harris (106) confirm Wolbach and Howe's explanation (328) that the gerüstmark is derived from osteoblasts in abnormal bone formation.

No satisfactory experimental study of the effects of ascorbic acid deficiency upon cartilage has been published, in spite of the opportunities offered in epiphyseal cartilage growth. Park et al. (232) present evidence that epiphyseal cartilage continues to grow in human scurvy after osteoid deposition has ceased. Unpublished studies by us and P. E. Boyle indicate that in prolonged periods upon greatly reduced ascorbic acid intake (0.3 mgm. to 0.5 mgm. daily) the epiphyseal cartilages of growing guinea pigs become defective, apparently due to loss of firmness of the matrix. Reparative sequences have not been studied in detail. The tardy and less striking responses of growing cartilage to the deficiency emphasize the well known differences between the matrix of cartilage and those of other supporting tissues.

The mechanism by which ascorbic acid promotes the formation of collagenous intercellular substances is not known. It may be involved in the chemical mechanisms (enzymes) of the cells responsible for the synthesis of this protein

product (collagen). The hypothesis that ascorbic acid or a derivative is part of the collagen structure cannot be excluded on the basis of present knowledge. Wolbach and Howe (328) found that in the destruction of tissues occasioned by operating upon a scorbutic guinea pig, substances were liberated which caused reparative changes in teeth and bones. This has been confirmed by Bessey and Boyle and has been shown to be too large an effect to be explainable by the small amount of ascorbic acid present in the damaged tissues, largely muscle (unpublished). This observation suggests that an anti-scorbutic factor (for whose synthesis ascorbic acid is necessary) may be in reserve in certain tissues. If so, it would explain the long period in man after disappearance of ascorbic acid from the blood and tissues required to produce the lesions of scurvy (71).

Tissue culture experiments have given conflicting results. Hass and McDonald (142) found that the addition of ascorbic acid to cultures of guinea pig fibroblasts did not promote collagen formation. They state that their "experimental data were in accord with the thesis of Wolbach and others that collagen is a secretory product of fibroblasts," although—"The observations failed to provide critical proof that this thesis is correct." v. Jeney and Törö had previously reported (155) that ascorbic acid was necessary for collagen formation in cultures of fibroblasts obtained from chick embryos.

Repair of wounds in guinea pigs in severe ascorbic acid deficiency is incomplete because of limited capillary formation and absence of collagen formation. The sequences of collagen deposition can be followed in such wounds following ascorbic acid administration and the relation of collagen to reticulum or argyrophil fibrils can be studied (333) (153). Wolbach (333) found that the first collagen deposited was homogeneous, then argyrophile fibrils appeared. The early argyrophilic fibril formation has been confirmed by Hunt (153) and Glasunow (111). The former refers to the argyrophile fibrils as precollagen and says that newly-formed collagen reverts to precollagen when the guinea pig is again put upon an ascorbic acid free diet.

The lesions of ascorbic acid deficiency in growing animals are mainly the result of the failure of formation of intercellular substances and the consequent weakness of supporting tissues. In the fully grown animal, lesions are much slower to appear and are mainly the result of failure of maintenance of intercellular materials. No one has succeeded in following the microscopic sequences of this failure in non-calcified tissues. In bone, osteoporosis makes the process visible; in other tissues, breaks accompanied by hemorrhages give evidence of the deterioration. The mechanical consequences in guinea pigs of long-continued inadequate supply of ascorbic acid are strikingly exhibited by the loosening and wandering of the teeth, the result of weakness of the collagen fiber suspending apparatus and of the alveolar bone (44).

In guinea pigs which received inadequate amounts of ascorbic acid (0.3 mgm. to 0.5 mgm. daily) for long periods, striking accumulations of connective tissue cells have been found, notably at attachments of muscle to bones and fascia (338). This may be interpreted as a compensatory hyperplasia, occasioned by mechanical weakness, the result of diminished collagen production.

Glasunow (111) in 1937 published fairly extensive studies of ascorbic acid deficiency in growing and in fully grown guinea pigs. His results agree with those of earlier publications. He characterizes scorbutus as a disability of all mesenchymal tissues in which they lose the property of differentiation. His account of sequences of bone resorption, osteoporosis in scorbutus, agree with those of Wolbach (333) in that an actual disappearance of bone matrix takes place with the liberation of bone corpuscles and fibrils. Ham and Elliott's (138) explanation is that in scorbutus there is failure to replace the normal breakdown, stating for a premise: "It is well known that the structure of bone is always changing; old Haversian systems constantly break down and are replaced by new. . . ." Hunt (153) for the first time observed the effect of deprivation of ascorbic acid upon newly formed scar tissue. He states that the new collagen reverts to "an argyrophil precollagenous state, very different from the comparable intercellular material in the scar of the control animal. . . ."

There are two views we may take in regard to the maintenance of intercellular materials. One is that the normal state is one of continuous breakdown and disappearance, with concurrent deposition of new materials; the other is that the normal state is one of equilibrium maintained by metabolic processes unaccompanied by physical breakdown. It is possible that new ideas may come from further studies of the effects of ascorbic acid deficiency. It has already been suggested (333) that the activities of osteoblasts may be reversible and bring about bone resorption. No important changes in blood vessels which can be attributed to ascorbic acid deficiency have been described, nor have morphologic changes been detected in capillaries. The capillary bleeding so common in scurvy is probably the result of structural weakness, either the result of changes in the cement substance binding the endothelial cells together, or in collagen fibrils immediately adjacent to the capillaries (74). New capillary formation is prevented by severe ascorbic acid depletion (333). Islands of hematopoiesis form adjacent to abortive capillary formations in the neighborhood of spontaneous hemorrhages or in the tissue surrounding blood clots after excision of muscle, in severe ascorbic acid depletion (334). The source of the blood-forming cells awaits demonstration, but the inference has been made that they are derived from endothelial cells which accumulate in consequence of failure of capillary formation (334).

Changes of importance in epithelial organs have not been proved to be a result of ascorbic acid deficiency. Atrophy of the adrenal, the result of depletion of lipids from the cortex, is a constant finding in long-continued depletion (24). Dalldorf (74) regards the keratosis of the hair follicles described by Aschoff and Koch (13) as probably due to vitamin A deficiency. The recent observation by Crandon, Lund and Dill (71) of the early appearance of similar lesions in a human subject on an ascorbic acid free diet, supplemented with "all other known vitamins," seems to prove that the epidermis is affected, particularly as the lesions disappeared after ascorbic acid therapy. Reparative proliferation of epidermis in acute severe ascorbic acid deficiency is not demonstrably impaired (328) (153).

Careful cytological studies of the tissues most markedly affected by ascorbic acid deficiency are much needed. Most of the studies of the distribution of ascorbic acid within cells, as revealed by silver nitrate reduction methods, have been made upon a few organs—adrenal, corpus luteum, interstitial cells of the testis, anterior pituitary and liver—and are reviewed by Bourne (40). Beyond changes in amount and distribution of ascorbic acid in cells, no cytological effect characteristic of the deficiency has been described.

The morphologic effects of ascorbic acid deficiency, like those of other vitamins, must be regarded as the result of retarded or suppressed normal activities of cells. In the growing animal, the first demonstrable effect is upon the formation of intercellular materials largely composed of "collagen." The deficiency affects quantity and quality of intercellular materials. Changes in morphology of the cells responsible for the production of intercellular materials may reasonably be regarded as reversible and as an expression of loss of a specific function. The qualitative changes of intercellular materials formed during the period of depletion and the mechanism of osteoporosis and resorption of matrices in general require further study and new techniques.

VITAMIN D DEFICIENCY. The vitamin D effect is the prevention or cure of rickets, a condition characterized by defective growth of bone, the result of retardation or suppression of normal growth sequences in epiphyseal cartilage and in calcification of bone and cartilage matrices. According to Bills (31) there are ten sterol derivatives having this effect. For clinical and experimental purposes, artificially activated ergosterol from plants and the naturally activated 7-dehydrocholesterol from fish liver oils have been used. Both are now obtainable in pure crystalline form.

The pathologic changes in rickets are the result of quantitative changes in the serum calcium and/or inorganic phosphorus content of the blood such as to retard or prevent calcification of cartilage, in a restricted region involved in growth, and of bone matrix. This decrease of calcium-ion and/or phosphate-ion below the critical precipitating level is in turn due to the very inefficient absorption of calcium from the intestinal tract in the absence of vitamin D (175). In infants, the lack of vitamin D alone is sufficient to produce rickets, while in a more resistant species such as the rat, the lack of vitamin D must be accompanied by a "relative deficiency of calcium or phosphorus or an absolute deficiency of either or both" (274). Similar accentuating conditions are probably necessary for the production of osteomalacia—the adult counterpart of rickets. The main action of vitamin D is to re-establish efficient calcium and phosphorus absorption and consequently to restore the concentrations of these ions in the blood plasma so that calcification can take place. There is no reason to believe that the cells and matrices concerned in bone growth and maintenance are defective in rickets or are directly acted upon by vitamin D (275). Bills has written an extensive critical review on the physiology of the sterols, including vitamin D (31).

The pathology of the faulty calcium and phosphorus metabolism which vitamin D corrects has for many years been well known. Important contribu-

tions to the pathology in humans are the publications of Pommer (241) and Schmorl (259). Erdheim (97) and Pappenheimer (223) made early important studies of experimental rickets. More recent publications have contributed relatively little to our knowledge of the tissue changes in vitamin D deficiency. On all facts of major importance there is general agreement. Most of the issues of today are those of interpretation of sequences and of details. Hess' book *Rickets, osteomalacia and tetany* (147) is a convenient source of information concerning human vitamin D deficiency conditions; that of Marek and Wellman, *Die Rachitis*, deals with spontaneous and experimental rickets in domestic animals (185). Both books have lengthy bibliographies. Goldblatt's 1931 review of experimental rickets has a bibliography of 2723 references (116).

Experimental rickets in animals duplicates completely the spontaneous disease in man and animals. The effects can all be explained as retardation of growth sequences in epiphyseal cartilage and of calcification of bone and cartilage matrices. The pathologic picture varies with the degree and duration of the deficiency (274) and in most spontaneous cases with the intermittent nature of the deficiency.

The sequences in endochondral bone formation which are disturbed in rickets are as follows: The epiphyseal plate of cartilage is firmly supported by bone on the epiphyseal side and uniformly penetrated by blood vessels of capillary dimensions on the diaphyseal (shaft) side. Growth is accomplished by continuous proliferation of cartilage cells, arranged in columns, on the epiphyseal side and concurrent degeneration of the matured cells on the diaphyseal side. The cavities occasioned by the degeneration and disappearance of the cartilage cells at the diaphyseal end of the columns are entered by capillaries accompanied by osteoblasts which form bone matrix upon the exposed cartilage matrix. The latter is calcified for a distance of two or three cartilage cells in advance (toward the epiphysis) of the entering capillaries. Endochondral growth of bone is thus achieved by a continuously retreating gap in the continuity of tissues, maintained on the epiphyseal side by continuous renewal of cartilage cells and on the diaphyseal side repaired by vascular outgrowth from the marrow comparable to repair of any defect of tissues by the process of organization or granulation tissue formation (274). The dependence of endochondral bone formation upon proliferation, differentiation, and degeneration ending in death and disappearance of the cartilage cells illustrates and epitomizes *cytomorphosis* in its four essential stages as defined by Minot (208).

The first evidences of rickets are the failure of the cartilage cells to complete the cycle of cytomorphosis and the failure of the matrix lateral to the persistent cartilage cells to calcify. In the absence of spaces created by the disappearance of cartilage cells there is no ingrowth of capillaries. Absolute rickets in the sense of the complete cessation of the above sequences is doubtful of achievement. The cessation takes place irregularly across the face of the cartilage plate. Wherever there is degeneration of cartilage cells, vascular ingrowth takes place, but the bone matrix (osteoid) deposited in the deficiency does not calcify.

The width of the epiphyseal cartilage continues to increase for a long period

because of the continued activity of the proliferative zone and the survival of the cells on the diaphyseal side. Deposition of bone matrix (osteoid) continues to take place around capillaries of the diaphysis adjacent to the cartilage. These two processes produce a zone of non-rigid tissue of abnormal width responsible for much of the skeletal distortion characteristic of the disease. In advanced rickets the non-calcified cartilage of the diaphyseal border is often transversely stratified, evidently a mechanical effect of weight-bearing. Osteoid which has accumulated is also molded by the pressure of weight-bearing. In long-continued rickets there is disappearance of the cancellous bone of the diaphysis and marked resorption of cortical bone.

The first histological evidence of repair following corrections of the diet is the presence of cleared or degenerated cells on the diaphyseal border of the cartilage, an effect visible at the end of 24 hours and accompanied by extensive vascular penetration within 48 hours. Calcification of cartilage matrix and of osteoid first takes place adjacent to capillaries which have entered spaces left by the degenerated cartilage cells, wherever this has occurred. Subsequently, the calcification of accumulated osteoid progresses toward the diaphysis. Cartilage matrix calcifies in proximity to capillaries. Excess osteoid which has accumulated during the deficiency is removed only after calcification. The removal is accompanied by many osteoclasts.

The above outline is based upon unpublished work by one of us and includes the sequences primarily occasioned by the deficiency. Dodds and Cameron (87) are in essential agreement with this account of the order of calcification and removal of osteoid in repair of rickets. We have omitted details, of interest only to pathologists, which are probably secondary to mechanical disturbances and certain consequent reparative sequences.

The facts generally accepted by investigators of rickets are: *a*, failure of calcification of the cartilage columns in the so-called zone of provisional calcification and failure of calcification of osteoid; *b*, continued growth and consequent increase in thickness of the diaphyseal cartilage and osteoid; *c*, lack of vascular growth into cartilage; *d*, resorption of bone formed before the deficiency.

Dodds (86) in normal endochondral ossification describes capillaries invading spaces occupied by cartilage cells which have not degenerated; some, he states, appear rejuvenated. Park (233) also describes capillaries invading normal cartilage cells and assigns an aggressive rôle to capillaries in endochondral bone formation and offers an explanation for the irregular penetration of capillaries too involved to present here. Harris (139) and Ham (133) as well as most textbooks, regard the cartilage cell as dead before capillary penetration takes place.

Our opinion, based upon the earliest changes to be seen in the repair of rickets, is that capillaries do not enter spaces occupied by viable cartilage cells and that the pattern of vascular penetration is determined by the distribution of cartilage cells which have completed their life cycle. The conditions necessary for this cycle are apparently the same as for those permitting calcification of cartilage and bone matrices.

An old question which has arisen from the study of rickets is whether cartilage cells transform into bone-forming cells. In the region where cartilage affected by mechanical pressure is in contact with osteoid—the rachitic metaphysis—appearances suggest that such a transformation does take place. Pappenheimer (223) avoided a discussion of the problem. Park (233) and Dodds and Cameron (88) are convinced that cartilage cells do change into cells which produce a tissue very much like osteoid. Pick (238) and Boucomont (38) describe a large scale transformation of cartilage into osteoid tissue. The evidence presented by Dodds and Cameron is worthy of careful consideration though amenable to another interpretation. In a study of normal and rachitic costochondral junctions, by means of very thin serial sections, we have not been able to obtain evidence of cartilage cells turning into osteoblasts. Bremer (47) (48) by reconstruction methods showed that in normal endochondral bone formation no osteoblasts are present in "closed lacunae which the primary marrow has not reached."

The initiation and repair of rickets in animals offer possibilities for the elucidation of some details of bone growth and maintenance, some of which have been pointed out in another place (334).

In osteomalacia (147) (139) (238) there is pathologic resorption of bone, presumably in response to needs of vital processes for calcium. There is abundant deposition of osteoid upon the remains of the bony trabeculae and in Haversian canals and upon the inner surface of cortical bone. Osteoblasts are very numerous and the layers of osteoid are very much thicker than in growing bone. The resorption of calcified bone and failure of newly formed osteoid to calcify are responsible for the yielding of the skeleton to normal stresses.

Teeth. The condition of rickets produces its most marked effect upon human teeth during the formative periods. Defective calcification of dentin and enamel and atrophy of odontoblasts and enamel organ resulting in hypoplastic teeth are consequences described (163). Essentially similar changes occur in the growing teeth of rats (23) (165) and guinea pigs (151).

Hyperplasia of the parathyroid glands in human and experimental rickets has long been recognized. A recent paper by De-Robertis (85) contains the bibliography of the subject. His own experiments with rats show that the hyperplasia is more marked in low calcium rickets whereas in low phosphorus rickets there is hyperplasia of the thyroid. The hyperplasia of both glands is accompanied by increased complexity of the Golgi apparatus.

Hypervitaminosis D. Irradiated ergosterol administered in excessive amounts produces untoward effects in man and animals (rats, mice, cats, rabbits, guinea pigs, dogs, monkeys and fowls). Preparations containing high percentages of toxisterol are most toxic. Irradiated ergosterol contains, in addition to the antirachitic factor, calciferol, small amounts of other pharmacologically active substances. Therefore, the possibility exists that some of the effects of feeding large doses of this preparation are due not to calciferol but to these related substances.

Crystalline calciferol (prepared from activated ergosterol) in sufficiently large doses is toxic and causes the characteristic lesions of hypervitaminosis D (31).

It is the only one of the ten forms of vitamin D (31) (32) that has been used in pure form for hypervitaminosis experimentation. The character of the lesions and to a large extent their pathogenesis is the result of the exaggerated physiological effect—hypercalcemia—of excessive dosage, hence other sterol derivatives having similar physiologic or pharmacodynamic properties probably are capable of producing the pathologic picture of hypervitaminosis D. We may regard this as proved for the vitamin D of cod liver oil (activated 7-dehydrocholesterol) from the review by Goldblatt (116) of early experimentation in this field.

For the physiologic aspects of this subject, the review of Bills (31) is recommended. The pathology up to 1931 is reviewed by Goldblatt (116).

Substantially all experimental hypervitaminosis D in animals and all of the few instances of human untoward effects have been produced by irradiated ergosterol. The lesions consist of disturbances of growth sequences and of maintenance of bone and metastatic calcareous deposition in many soft tissues. Necroses in liver, kidneys and heart muscle may occur even though calcareous deposits are slight or absent (269). Whether or not degenerative changes always precede and determine the sites of calcareous deposits has not been definitely determined.

Bone changes. In conformity with the pharmacodynamics of activated ergosterol, there is variation with dosage, dietary calcium and phosphorus and age or rate of growth of the animal. The first effects of hypervitaminosis D in growing animals is upon endochondral bone formation. Moderately excessive doses of activated ergosterol accelerate or exaggerate sequences normal to bone growth at the epiphysis. Calcification of the provisional zone of calcification of the cartilage proceeds at an accelerated rate and the bones becomes hypercalcified (269) (275) (51). The narrowing of the epiphyseal cartilage plate and the engorgement of the capillaries penetrating the empty cartilage cell spaces (140) (258) may well be regarded as a speeding up of the cytomorphosis of cartilage essential to endochondral bone formation (334).

Larger amounts of vitamin D retard growth, presumably because of failure of growth of cartilage. The bone trabeculae which continue to form do not calcify completely, although some calcification continues apparently at the expense of cortical bone which undergoes osteoporosis (51). The degree of these changes and rate of progress are influenced by the dietary calcium and phosphate intake, shown independently by Harris and Innes (140) and Shelling and Asher (269), because the determining factor is maintenance of the elevated serum calcium level imposed by the dosage of vitamin D administered. Phosphate retention brought about by bilateral nephrectomy, accelerates the appearance of hypercalcemia and bone resorption, both of which may appear in 48 hours after excessive doses of calciferol (202). Vitamin A deficiency, because of its effect upon the kidney (keratinizing metaplasia in pelvis and ureter) as well as subtotal nephrectomy also accelerates the deposition of calcareous deposits (221).

Ham and Lewis (137) succeeded in administering to rats fed on "stock laboratory diet" amounts of activated ergosterol which produced short bones, non-

calcified trabeculae of unusual thickness and number at the epiphyseal ends, resorption of cortical bone and non-calcified matrix deposition of periosteal and endosteal origin. The growth (epiphyseal) cartilage was narrower than normal. Because of the accumulation of non-calcified matrix at the sites named, they regarded the condition as rachitic in character, disregarding the lack of the cartilage changes characteristic of rickets (274). Changes practically identical to those of Ham and Lewis had previously been described by Harris and Innes (140) who also noted that the "resorption of bone is much lessened when the diet is rich in calcium". In advanced degrees of hypervitaminosis D, resorption of bone is the most prominent feature. The most marked degree of bone resorption occurs on calcium-free diets or on diets rich in phosphorus and deficient in calcium (140) (269). Resorption of bone is of the osteoporotic type and accompanied by osteoclasis. Shelling, Asher and Jackson (270) described the relation of the bone changes to the hypercalcemia and hyperphosphatemia induced by parathormone administration in rats. These, in regard to dosage and dietary conditions, paralleled those of hypervitaminosis D. One outstanding difference was that the regions of bone resorption produced by parathormone were replaced by fibrous tissue. In general, however, the effect on bone seems to be a consequence of hypercalcemia and influenced by the dietary intake of calcium and phosphates. The comparisons by Shelling and Asher (269) (270) of the effects upon bone of excessive parathormone and vitamin D dosages in relation to calcium and phosphorus metabolism are important contributions to the histo-physiology of bone. For details of bone lesions produced by hyperparathyroidism and a comparison with those of hypervitaminosis D, consult Jaffe's review (154).

The effects of hypervitaminosis D of pregnant rats upon the bones of the offspring have been studied by Collazo, Rubino and Varela (63) and Shelling and Asher (269). The important consequences are cessation of cartilage growth and hypercalcification of bone, comparable to the effects described in rats with abundant calcium intake upon moderate over-dosage with activated ergosterol.

Teeth. Harris and Innes (140) described excessive calcification of dentin in teeth of rats and an extraordinary overgrowth of cementum. Weinmann (317) described in rats, hyperplasia and hypercalcification of both primary and secondary cementum, which were somewhat similar to the changes in bone (alveolar).

Schour and Ham (261) found that both parathormone and vitamin D in single massive doses resulted in the formation first, of a strip of dentin which was imperfectly calcified and, second, of a strip of dentin which was normally or excessively calcified. Their results, they thought, could be explained by the theory that vitamin D acts through the parathyroid mechanism.

Wilton (325) has described changes in endochondral bone formation in a human case of hypervitaminosis D which were essentially the same as those described in animals.

Lesions and metastatic calcification in soft tissues. Calcareous deposition in many tissues is plainly the consequence of hypercalcemia and, in the circumstance of abundant calcium intake, occurs before bone resorption takes place. Activated ergosterol is more potent ("toxic") in this regard than the vitamin D of fish

liver oil (activated 7-dehydro-cholesterol) (209) (141). Kidneys and blood vessels have received most attention. There are no distinctive features of the necroses (uncalcified) which have been described in liver, kidneys, and heart muscle.

The kidney, presumably because of excessive urinary secretion of calcium (119) (288) (246) is most liable to calcareous deposits. Degeneration of the kidney tubules with calcareous deposits in the lumen, within degenerated epithelial cells and between the tubules and stroma is the most constant feature. Calcification around the glomeruli and of the renal blood vessels is common (119) (283) (59). Too little attention has been paid to the exact location and nature of the deposits. According to Spies and Glover (283) in rabbits the basement membranes of tubules and glomerular capsule become thickened and hyalinized before or concurrently with the calcification. The deposits outside of tubules usually are discrete, laminated, and lobulated. Appearances after decalcification suggest the presence of incorporated organic material (59) (338).

The blood vessels of the kidneys are liable to calcification and Spies and Glover (283) are inclined to attribute the tubular degeneration to vascular lesions. Gough, Duguid and Davies (119), Steck et al. (288), Chown et al. (59) and Vanderveer (309) regard degeneration of the tubules as the result of a toxic consequence per se.

Practically all experimenters with hypervitaminosis D have described calcification of the blood vessels. Spies and Glover (283) found lesions in arteries and veins of the rabbit's kidney. In the former the calcareous deposits were chiefly in the media and internal elastic lamina and accompanied by hyalinization of the media. Duguid (89) made a careful study of the rat's aorta in hypervitaminosis D, and said that the lesion consists of a muscular degeneration with calcification of the media. Apparently he regarded degeneration of smooth muscle cells as a primary effect. Calcification took place first in elastic laminae, and extended into the degenerated tissue between them, but also extended into non-degenerated tissue. Duguid believes that the degeneration of smooth muscle is a probable consequence of the disturbance of calcium metabolism but does not require calcium deposition in situ. He described reparative proliferation, over calcareous deposits, of the intima with plaque formation. Vanderveer (309) is convinced that degeneration precedes calcification in blood vessels and other organs. His description of the rabbit's aorta in hypervitaminosis D corresponds closely with Duguid's for rats, though he thinks, contrary to Duguid, that the proliferation of the intima which is less common in the rabbit, is not secondary to lesions of sub-intimal tissues. He noted degenerative lesions followed by calcification in kidneys, stomach (muscle), heart, liver and lungs. Ham (134) found that single large doses of irradiated ergosterol will produce massive calcification in the aorta, coronary vessels and heart muscle, as early as 48 hours after administration. Microscopic sections of tissues of similarly treated rats made after 24 hours "showed nothing that would presage such an imminent catastrophe, so that calcifications do not appear to depend on degenerative changes in the recipient tissue." He found calcium deposits on elastic fibers and in degenerated and necrotic smooth muscle. Ham and Lewis (136) reaffirm the absence of degenera-

tion preliminary to calcification in the coronary vessels of the rat's heart. They state that the media calcifies first and is followed by proliferation of cells of the intima.

In general there has been insufficient precise histological study of the pathologic sequences in hypervitaminosis D. All workers are in agreement that calcareous deposits are secondary to hypercalcemia but there is disagreement concerning a direct toxic action of vitamin D upon tissues not related to the effect on the blood calcium level. Ham and Portuondo (135) found that after single massive doses of activated ergosterol, pathologic calcifications did not appear when the serum calcium was rising, but did appear in large amount when it was falling. Cowdry and Scott (69) in monkeys, after using amounts of viosterol far in excess of therapeutic dosages but not sufficient to materially affect serum calcium and phosphorus levels, found few lesions in blood vessels and kidneys not found in the controls. Their results are inconclusive for premises in the consideration of mode of action of the vitamin on tissues. Goormaghtigh and Handovsky (118) studied various degrees of hypervitaminosis D_2 (calciferol) in the dog with particular reference to renal arterioles and in particular to the juxtaglomerular neuromyoarterial apparatus. Moderate doses caused hypertrophy of both the afibrillar (sensory leiomyoblasts) and ordinary smooth muscle cells of the latter, which they attribute to a stimulation of metabolism by calciferol. Larger doses of calciferol over longer periods caused atrophy and even necrosis of smooth muscle cells but not of the afibrillar muscle cells. Arterioles and prearterioles showed degeneration of the smooth muscle of the media. Calcification they regarded as a secondary feature in the vascular lesion of hypervitaminosis D. The effect upon the juxta-glomerular apparatus, in their opinion, is a factor in the production of elevated blood pressure in hypervitaminosis D. This paper presents the strongest evidence thus far produced that massive doses of calciferol have a specific toxic action other than the effect upon calcium and phosphorus metabolism. Chown et al. (59) in addition to calcification in internal organs noted transitory calcification of the skin of the head with baldness in young rats, but did not make microscopic examinations.

A specific toxic effect of excessive vitamin D administration upon the cells of parenchymatous organs remains an unsettled question. In spite of the affirmatory evidence at hand there remains the doubt which is raised by the many similarities to be found in the effects produced by experimental hyperparathyroidism. The tendency to regard tissue changes other than calcification as the result of alterations in cell environment in the period of ascending hypercalcemia is a tempting one. Knowledge of the precise location of the initial calcium deposits in soft tissues would be of considerable value. From such evidence as can be found in the literature, it is between cells. Further exploration of the problem may yield information regarding a possible rôle of intercellular materials as a temporary repository for materials which diffuse from blood vessels in maintenance of homeostasis.

VITAMIN E. For a full account of the development of evidence leading to the establishment of vitamin E as a nutritional essential, the isolation of the active

compounds, alpha, beta and gamma tocopherols from wheat germ and other plant oils, the chemistry and synthesis of these new substances and what is known of their physiology and distribution in nature, the reader is referred to recent excellent reviews elsewhere (100) (195) (16) (201) (281) (278).

Pathological lesions preventable by pure vitamin E (tocopherols) have been demonstrated by: *a*, failure of early embryonic development in the rat (98) (308) (101) (95) (193) and mouse (192); *b*, irreparable degeneration of germinal epithelium in the rat (98) (193) (186) (103); *c*, a degeneration (nutritional dystrophy of the skeletal musculature in guinea pigs, rabbits (112) (215) (273) (178), rats (99) (220) (20) (113) (93) (102) (166), dogs (10), and ducklings (226); and *d*, a nutritional encephalomalacia in the chick (225) (76) (227). In addition, degeneration of the testes of male fowls (2), early embryonic death of chicks (1), an idiopathic degeneration of the smooth musculature of the young turkey (164), and severe spinal cord lesions in adult rats (93) have resulted from feeding vitamin E low rations, but not yet shown to be preventable by tocopherols. Evidence of the need for the vitamin in other species is indirect and controversial. It consists of reports of improvement in reproduction (human, cattle, pigs) in cases of repeated spontaneous abortion, exclusive of that due to pathologic states of the uterus (313) (276) (68) (30) and of contradictory reports of benefits from treatment with alpha tocopherol in human neuromuscular and muscular dystrophies (316) (268) (199). Because of the wide distribution in natural foods (cereals, green vegetables, etc.) the deficiency of this factor has been considered as improbable in human nutrition; however, knowledge of requirements and possibilities of conditioned deficiencies due to faulty absorption or metabolism (50) remain uncertainties. Obviously, the value of vitamin E to man is not established and is in need of well-controlled investigation.

Reproductive failure in vitamin E deficient rats has been carefully studied by Evans and Burr (98) and Urner (308). Estrus, ovulation and implantation are normal, as is the morphology of the ovary and uterus. Death of the developing embryo and its resorption occur probably because of starvation and asphyxia as a consequence of limited development and failure of function of the placenta. Retarded fetal development becomes evident soon after implantation (7th to 10th day of gestation) by delay in appearance of the ectodermal cavity followed by underdevelopment and rarefaction of all the fetal tissues, especially those of mesodermal origin. The yolk sac shows a reduction in size and number of endothelial villi and blood islands. The outgrowth and differentiation of the allantois, its extension toward the trophoblast and the development of the fetal placenta are retarded, thus resulting in a delay or failure to establish maternal-fetal circulation. The death of the fetus (12–14 days) is followed by necrosis and resorption. The maternal placenta is smaller than usual and shows some distention of vessels, but is not greatly altered and continues to grow for a few days after fetal death, before regression. The uterus returns to normal, usually by the 25th day. According to Adamstone (1) the low hatchability of eggs from vitamin E deficient hens is also due to early embryonic death. In the chick, underdevelopment of fetal tissues, hemorrhage and the formation of a mesodermal

ridge ("lethal ring") around the blastoderm which chokes off the vitilline circulation to the yolk sac, causing lack of nourishment and death, is apparently the order of events.

While in rats maternal tissues, including ovaries, escape lasting damage in spite of the loss of the developing fetus, in the male the germinal epithelium becomes completely and irreparably degenerated. Vitamin E restores fertility to a few animals only, even if administered at the time of the first morphological evidence of testicular degeneration (98) (193) (191). Detailed histopathological descriptions of the testicular degeneration, first reported by Mattill (100) have been furnished by Mason (188) (191) and Evans and Burr (98). There is early loss of motility of spermatozoa and then progressive degeneration of the entire germinal epithelium beginning with the most mature cells. Fusion of the sperm, extensive nuclear chromatolysis of spermatids and secondary spermatocytes (which tend to coalesce and form giant cells) followed by nuclear and cytoplasmic degeneration of the primary spermatocytes and spermatogonia is the sequence of events, according to Mason (188). The atrophic seminiferous tubules become lined only with Sertoli cells. The interstitial cells remain structurally and apparently functionally normal, as indicated by normal gonadal hypophyseal relations. The accessory sex glands are apparently unaffected by vitamin E deficiency. According to Evans and Burr (98) the number, morphology and motility of sperm in the ejaculate are normal in the earliest stage of the development of functional sterility; at this stage loss of fertilizing power is made evident by mating experiments.

Testicular degeneration in cocks resembling that in vitamin E deficient rats has been reported after prolonged maintenance (2–3 years) on a ration treated with ferric chloride in order to destroy vitamin E (2).[2]

Reparable sterility as the result of degeneration of the more mature cells only of the seminiferous epithelium or of resorption of the developing embryo, may be caused by inanition or the absence of other dietary factors—such as vitamin A, which influences growth and impairs health. Mason (191) has recently reviewed this whole subject.

Although there have been numerous reports of physiological and pathological evidence relating vitamin E to the pituitary, thyroid and other glands of internal secretion, the weight of contradictory evidence makes it clear that no constant and direct relation has been established (195) (16) (201) (191) (167). Some vitamin E deficient male rats show a change in the basophilic cells of the anterior hypophysis analogous to that found in cryptorchidism and castration, but the nature of the change and the fact that the female pituitary remains normal supports the conclusion that this mild change is secondary to the testicular degeneration. The hypoplastic changes of the thyroid leading to cretinism, reported by

[2] This procedure has been developed in an effort to supply vitamin E deficient rations which are adequate in other respects and satisfactory for use with birds and other animals which do not do well on the purified diets. Although this leads to a diet which will produce sterility in the rat, there remain uncertainties both regarding its freedom from vitamin E and its completeness as a ration.

Barrie (19) have not been confirmed by Evans et al. (103) and Pappenheimer (228) as a constant feature of vitamin E deficiency. Mason (191) has recently reviewed the literature on the above points, and suggests the wisdom of considering slight and inconstant changes in other glands of internal secretion as secondary to the reproductive sterility until experiments prove otherwise.

Generalized severe degeneration of the skeletal muscles without emaciation or obvious lesions in other organs in guinea pigs and rabbits, as a result of vitamin E deficient diets, was first reported in 1931 by Goettsch and Pappenheimer (112) who, however, did not regard vitamin E deficiency as the cause. Nutritional muscular dystrophy, now known to be an effect of vitamin E deficiency, has been produced in several species and studied by a number of workers. Extensive references are contained in recent reviews by Evans (104), Pappenheimer (230), Mattill (197) and Morgulis et al. (215). Protection of guinea pigs (273) and rabbits (178) with alpha tocopherol does not support the contention of Morgulis (215) that more than one factor is involved. The inclusion of cod liver oil or rancid fat in vitamin E deficient diets increases the severity of the muscle lesions in guinea pigs, rabbits and goats, apparently the result of destruction of vitamin E in the diet, gastro-intestinal contents, or tissues, although a direct toxic effect upon muscle has not yet been excluded (77) (184) (196) (179) (273).

The severe paralysis with subsequent death or spontaneous recovery of apparently well nourished suckling young rats of mothers on low E diets, first observed by Evans and Burr (99), is the result of muscle lesions identical with those in the guinea pig and rabbit (112) (220) (228) and are preventable by alpha-tocopherol administration either to the new born young or the lactating mother (20) (113). Nutritional muscular dystrophy in older rats follows a more chronic course. Paralysis, developing over a period of several months and varying in severity from mild incoördination and ataxia to almost complete incapacity has been observed by numerous investigators (93) (102) (177). Early stages of the disease, before paralysis is evident, are detectable by a decreased capacity for muscular tension, lowered muscle creatin and scattered necrotic fibers (166). Severe muscular atrophy finally becomes grossly evident. The animals may live for months in an almost helpless condition. Wheat germ oil or alpha tocopherol will prevent the disease or halt the progress (93) (177). Cures have been reported when treatment was prolonged and instituted before gross paralysis was evident (166).

A vagary in distribution should be mentioned. In guinea pigs (112) the masseter and tongue muscles escape although all other voluntary muscles are affected. In rats (228) the tongue muscle alone escapes although the masseter is not constantly affected.

The pathology (112) (228) (166) of the muscle degeneration is essentially that which in human pathology has long been known as hyaline, waxy, or Zenker's degeneration and associated with infectious diseases such as typhoid fever and the pneumonia of epidemic influenza. The sequences of the muscle fiber lesions in vitamin E deficient animals as described by Pappenhemier, including reparative regeneration of incompletely degenerated fibers, are essentially the same as those described in muscle from persons who died in consequence of epidemic influenza

with pneumonia (327). The lesion is a rapidly produced necrosis of muscle fibers characterized by conversion of a part or the whole of individual fibers into hyaline structureless necrotic material, which breaks up into globular and irregularly fragmented masses. If the sarcolemma survives regeneration occurs; otherwise the sequences are those accompanying necrosis from diverse causes. In the rat the preservation of detail in necrotic fibers seems to be more common than in human muscle and calcification occurs more often. We regard the as yet undetermined (228) disturbed physiology responsible for the lesion and its correlation with the factors concerned with Zenker's degeneration in the human to be of first interest rather than the apparently non-specific nature of the morphological sequences. Facts (228) (230) of importance for premises toward a physiological explanation are: *a*, that the similarity of the lesion to Zenker's degeneration of infectious diseases in man suggests a toxic product of deranged metabolism as a cause; *b*, that muscle fibers in proximity to the blood vessels of the fascia are more apt to escape than those more remote; *c*, that unilateral section either of the sciatic nerve or of the Achilles tendon gives unilateral protection, whereas inactivation of a limb by a cast does not, and *d*, transection of the lower spinal cord has given inconclusive results.

The myocardium and the smooth muscles of the intestine and all other organs are unaffected in rabbits and suckling rats. The brain, cord, peripheral nerves, terminal nerves and end plates were well preserved (228). Olcott (220) and Barrie (20) also found a normal nervous system in acutely dystrophic rats. Einarson and Ringstead (93) have described severe cord and peripheral nerve lesions preventable by wheat germ oil in their older vitamin E deficient rats, and compare certain stages of the disease (degeneration of pyramidal tract, anterior cells and dorsal sensory tracts) with amyotrophic lateral sclerosis and progressive muscular dystrophy, two important degenerative diseases in man.

Limited observations by Wolf and Pappenheimer (339) apparently confirm the occasional presence of cord lesions in the chronic deficiency. On the basis of present pathological knowledge and the contradictory clinical reports (30) (316) (268) (199) one must conclude that no relation has been established between alpha tocopherol and the neuromuscular dystrophies of man.

Paralysis due to nutritional encephalomalacia is the consequence of vitamin E deficiency in newly hatched chicks (225) (227) (4) (339). The essential lesion is an ischemic necrosis followed, if the animal survives, by reparative organization of the dead tissue. The cerebellum is most severely affected but the cerebral hemispheres, medulla and mid-brain may suffer milder injury. The vascular origin of the disease is indicated by the physiological demonstration of impaired blood supply to the cerebellum before lesions are evident, the presence of thrombi and the histopathology of the lesion. The following descriptive summary of the lesion has been given by Pappenheimer, Goettsch and Jungherr (227) "a degenerative lesion, characterized by edema, rapid necrosis of the neural elements, and later of the astrocytes and oligodendroglia. As reaction changes, one may list the multiplication and local increase of the microglia and subsequent transformation into compound granular cells, the proliferation of endothelium with the formation of new vascular ingrowths into the degenerated areas, and finally, the

partial mesodermal organization of the softened tissue. The disease is difficult to produce in chicks more than a few weeks old but may occur in the young in varying degrees of severity from mild lesions demonstrable only by section and causing no symptoms, to those involving most of the brain, easily detected grossly and resulting in severe paralysis and death. Spontaneous unexplainable recoveries are frequent."

The turkey reacts to vitamin E deficiency by a degeneration of the smooth musculature of the gizzard, while the skeletal musculature remains apparently normal[3] (164). There develops a patchy hyaline necrosis of the smooth muscle fibers, accompanied by an inflammatory reaction, followed by fibrosis and attempted muscle regeneration. Ducklings respond to the same diets by developing a severe myopathy analogous to that found in mammals (226) (29). It is interesting to note that corresponding diseases have been found in chicks, turkeys and ducklings obtained from commercial hatcheries (227). Species variation is further emphasized by the fact that adult rats protected from muscle dystrophy may nevertheless be sterile (193) while dystrophic young have been born and the testicular epithelium found normal in rabbits with severe muscle dystrophy (230) (180). Another example of species variation is the occurrence of muscular dystrophy in the mouse without testicular degeneration (231). The influence of physiological state on the manifestations is shown: *a*, by the acuteness of the muscle dystrophy of young rats (99) (113) as compared with old (102) (166); *b*, the reproductive requirements of the male compared with the female rat (193) (95), and *c*, the refractivity of older chickens and turkeys (225) (164). Adamstone reports a variety of lesions occurring in chicks raised on the iron-treated diets with liver oil supplements—lymphoblastomas (3), erythrophagocytosis (5), reticulum cell sarcomas (6). These unconfirmed effects are difficult to evaluate because of the involvement of factors other than vitamin E.

The striking variety of the pre and post-natal pathological manifestations of vitamin E deficiency indicates the need for further efforts to find a common morphological or cytological characterization. The explanation of spontaneous recoveries in young rats and chicks remains a mystery. The relation of cod liver oil and rancid fats to experimental dystrophy and vitamin E should continue to receive attention. Further studies of the pathology of nutritional muscular dystrophy relative to nerve changes and the neuromuscular diseases of man are of obvious importance. Does man require vitamin E? If so, how much, and what are the manifestations of the deficiency—muscular, reproductive, or other disturbances?

VITAMIN K. No contribution to the pathology of vitamin K deficiency has been made since Dam and Schönheyder's description in 1934 (75) of "A deficiency disease in chicks resembling scurvy." In addition to hemorrhages occasioned by slight traumata, they described erosions of the lining membrane of the gizzard. Another, at present unidentified, factor is now held to be responsible for the gizzard lesions (8) (9).

[3] This is to be distinguished from the gizzard erosion occurring in chickens and other birds apparently also due to the absence of an essential nutritive factor.

We have been unable to find any description of the pathology associated with vitamin K deficiency in birds or mammals which mentions more than the occurrence of hemorrhages. It is not clear that hemorrhages occur without some degree of trauma. We wonder if diminished clotting power of the blood is the complete explanation of the bleeding because it requires the assumption that in ordinary activities, with attendant minor traumatizations, the clotting mechanism is constantly being called into action in normal individuals. If a low prothrombin level does have an effect upon the vulnerability of capillaries, it is doubtful if the microscope can reveal it.

It is now known (244) (49) that sweet clover disease of cattle, in spite of the hemorrhages (260) (253) (254) is not a consequence of vitamin K deficiency *per se*. The mechanism of the prothrombin deficiency in this disease has not been worked out.

Extensive bibliographies pertaining to vitamin K are contained in the reviews by Almquist (9) and Brinkhous (49) and in the book by Butt and Snell (53).

VITAMIN B COMPLEX. Realization of the presence in certain natural products (yeast, milk, liver, etc.) of nutritional factors essential for certain mammalian and avian species and their common absence from rations usually employed in experimentation led to the association of a group of otherwise unrelated substances under the indefinite but widely used term "Vitamin B Complex." Contemporaneous knowledge of the factors comprising the "B complex" and the highlights of the historical development of the subject are to be found in the recent book by Eddy and Dalldorf (92) and in chapters by Nelson (219) and Sebrell (266) in the American Medical Association monograph on vitamins. Space limits this review to a brief summary of the pathology of only the best known members of this group of substances.

THIAMIN (VITAMIN B_1) DEFICIENCY. The striking manifestations of disturbed physiology of acute thiamin deficiency are accompanied by no, or at most, a few apparently simple tissue changes. Degeneration of peripheral nerves, so generally accepted as its outstanding consequence, has been shown experimentally to be a result of a more chronic course of this deficiency. Another feature of spontaneous thiamin deficiency in man (beri-beri) which also has been duplicated in animals is cardio-vascular failure accompanied by right-sided enlargement of the heart, venous congestion of the abdominal viscera, effusions into serous cavities, and edema of the extremities. Paraventricular degeneration and hemorrhage (Wernicke's disease in man) has also been reproduced in thiamin deficient pigeons and rats.

Access to the literature, historical and experimental, may be had through the bibliographies of a few comprehensive publications; the books of Williams and and Spies (324), Eddy and Dalldorf (92) and papers by Cowgill (70) and Vedder (311). A recent discussion of the cardiovascular manifestations of thiamin deficiency in man and animals with a good bibliography is that by Weiss (318). An interesting recent human experimental study is reported by Williams, Mason, Wilder and Smith (321).

Thiamin requirements are proportional to the available carbohydrate used by

the organism (324) (70) (296). Therefore, the course of the deficiency can be influenced by either of these two variables. Likewise, unless efforts are made to maintain adequate caloric intake, starvation may become a compensating factor in preventing the development of typical symptoms and lesions, or in making the deficiency more chronic in appearance (296). Most of the pathologic physiology and pathology of thiamin deficiency is undoubtedly explainable on the basis of the rôle of thiamin in carbohydrate metabolism. The marked involvement of the nervous system is significant "when one considers that nervous tissue with respiratory quotient of unity is totally and uniquely dependent upon oxidation of carbohydrate for its function and integrity" (321).

Swank (295) and Swank and Bessey (296) emphasize the variation in symptoms and lesions of thiamin deficient pigeons according to the severity and duration of the deficiency.

Careful studies by Swank and Prados (297) on acutely deficient pigeons showed that selective mild degeneration of the peripheral portions of many axons from the vestibular nucleus was frequently but not invariably present in early opisthotonus, while the proximal portions of the axons and the cell bodies remained histologically normal. The earliest changes which could be recognized were swelling and deep staining of the neurofibrils in the portion of the axon involved. However, the functional origin of the opisthotonus seems evident from the rapid response (few hours) to replacement therapy. No other nerve lesions of significance were observed in the acute deficiency. Swank and Bessey (296) have found that rations inadequate in thiamin (chronic deficiency) in pigeons is followed not by opisthotonus but by leg weakness and paralysis, with degeneration of the peripheral nerve which begins at a point most distal to the cell body and progresses centrally. Thiamin therapy, if instituted before neuron death, results in recovery in a time commensurate with the known rate of axon regeneration. These conclusions were based on observations made at different levels of the sciatic nerves and the employment of suitable techniques for the study of axis cylinders and myelin sheaths. Adequate controls excluded the possibilities that these effects were due to general inanition or absence of other factors. According to Swank (295) myelin sheath changes follow those in the axis cylinder. Zimmerman (344) (345) holds the contrary view.

Prickett (242) and Prickett, Salmon and Schrader (243) found, in acutely thiamin-deficient rats, no more degenerated nerve fibers than in normal controls. In chronically deficient rats, marked degeneration of nerves was found and long-continued deficiency caused irreparable damage. Peripheral nerves only were studied by means of polarized light and Sudan III techniques upon frozen sections, so that it was not proved that the irreparable damage (paralysis of the rats) was the result of death of neurons.

In dogs also, Street, Zimmerman, Cowgill et al. (291) found that the signs of nervous origin (vomiting, stiffness, and unsteadiness of the hind legs) in acute thiamin deficiency disappeared within a few hours after administration of the vitamin. They regarded the early damage to the nervous system as functional. In chronically deficient dogs, recovery did not take place in a month during

which time large amounts of thiamin were given. No mention is made of the pathology of the acutely deficient dogs, but in the chronically deficient ones there was myelin degeneration of peripheral nerves and the posterior columns of the spinal cord. In two dogs there was gliosis in the posterior columns and in addition, in one dog, there were bilaterally symmetrical glial scars in the dorsal spinothalamic tracts, proof of irreparable injury. It seems clear that thiamin deficiency *per se* can lead to nerve degeneration. Other deficiencies which also may result in nerve damage are discussed elsewhere.

Bilaterally, symmetrical minute hemorrhagic lesions in pons, medulla, and cerebellum, in the brains of thiamin deficient rats and pigeons were first described by Prickett (242), later by Church (61). Zimmerman (345) and Alexander (7) from comparative studies of the lesions in the brains of pigeons and humans, have come to the conclusion that thiamin deficiency is the cause of Wernicke's disease in man. This disease is associated with chronic alcoholism and characterized by minute hemorrhages into nuclei around the ventricles, most constantly involving the nuclei of the extrinsic muscles of the eye (7). Alexander (7) explains the lesions on the basis of "angiodegeneration with varicose deformity of the vascular bed" and states that: "The resulting subacute necrosis of the parenchyma with glial proliferation and proliferation of endothelial and adventitial elements of the vascular walls, as well as the hemorrhage, are secondary." Swank and Prados (297) review the subject of cerebral lesions and on the basis of their experiments upon pigeons and cats come to the conclusion that the hemorrhages are secondary to degenerative changes in the neurons surrounding blood vessels. Proliferation of adventitial cells of blood vessels and gliosis they regard as reparative responses. They suggest that the vascular changes are the result of accumulation of acid metabolites in the adjacent tissues. The sequences they describe are vascular hyperemia, perivascular edema and, finally, hemorrhages.

A wholly satisfactory explanation of the hypertrophy of the right side of the heart and eventual left-sided failure has not been achieved. The edema of beri-beri and serous effusions in thiamin-deficient animals are in part to be explained by the disturbed physiology of heart and peripheral blood vessels. These subjects are discussed by Vedder (311) and Weiss (318). Swank and Bessey (298) have found that the characteristic manifestations of cardiac failure (beriberi) can be regularly produced in the pigeon by chronic thiamin deficiency.

Numerous observers have reported hypertrophy of the islands of Langerhans in thiamin-deficient animals (335).

There is no lesion specific for thiamin deficiency in the sense that widespread keratinization of epitheliums is characteristic of vitamin A deficiency and the effects upon intercellular materials is characteristic for ascorbic acid deficiency.

The inability of cells to utilize carbohydrate sufficiently for the needs of normal processes may alone be responsible for the degeneration of neurons. Accumulation of acid metabolites has been a favorite theory in accounting for the lesions. We recommend Cowgill's (70) discussion of the physiology of thiamin. Possibilities for the investigation of some aspects of neuro-physiology may be found

through study of thiamin deficiency states, as suggested by the early neurological responses and the pathology.

RIBOFLAVIN DEFICIENCY. Riboflavin (lactoflavin), once called vitamin B_2 or G, is known to be a nutritional essential for growth and normal health for the rat (148), mouse (125), chick and turkey (172), pigeon (125), dog (263) (290), pig (152) (235) and man (302), and because of its widespread distribution and fundamental rôle in cell respiration is probably required by all vertebrates and by many lower forms (22) (280). General knowledge of the subject, methods of isolation, synthesis, function in cellular oxidation-reduction enzymes systems, human requirements, distribution in foods and relation to other members of the "B group" of vitamins can be found in recent reviews and textbooks (272) (200) (37) (271) (17) (21) (267) (217).

Lesions of the eyes, skin, mouth, tongue, and nervous systems and profound collapse with coma and death have been reported in more than one species as manifestations of riboflavin deficiency. The only histopathological studies of uncomplicated riboflavin deficiency are on the rat (92) and dog (263) (290) (292), and these are limited and sketchy. However, it seems reasonably certain from the similarity of gross appearances and other considerations that the primary tissue alterations are the same in most species.

The ocular signs and symptoms of riboflavin deficiency in both man and experimental animals are photophobia, contraction of the palpebral fissure, lacrimation, burning and itching of the eyes, soreness and swelling of the lids, visual fatigue, blurred vision, congestion of the conjunctiva and limbic plexus with ingrowth of capillaries into the cornea, and keratitis with eventual ulceration (302).

The experimental pathological studies in the rat by Bessey and Wolbach (26) and the subsequent clinical and experimental observations by Kruse, Sydenstricker, Sebrell and Cleckley (168) (301) and others (91) (284) (150) (158) definitely established that the corneal symptoms and lesions are characteristic of riboflavin deficiency and the earliest and most easily recognized diagnostic sign.

Our histological and slit-lamp studies of the developing corneal lesions in the rat showed that the "sprouting" of capillaries from the limbic plexus into an otherwise normal cornea was the first morphological indication of riboflavin deficiency. The first invading capillaries lie just beneath the epithelium and extend toward the center of the cornea. Later they penetrate the tunica propria. As the deficiency progresses, edema, cellular infiltration and separation of the fibers of the tunica propria, with resulting corneal cloudiness, appear. The corneal epithelium remains grossly unchanged until late in the deficiency and then undergoes degenerative changes which are regarded as secondary to conditions in the tunica propria. In advanced cases, the collagen fibers of the tunica propria become fused and hyalinized and newly formed fibroblasts appear in the zone of capillary ingrowth and infiltration. The superficial epithelium over such areas becomes markedly changed and separated from the deeper layers. Necrosis and ulceration may follow. Prompt regression of all lesions follows ribo-

flavin administration. The cornea may become clear and a diminished blood flow through the newly formed capillaries becomes evident within a few hours; and unless ulceration has occurred, the eye appears histologically normal within a week, except for the presence of collapsed capillaries, demonstrable only by injection methods. Slit-lamp observations and studies on man in 1941 indicate an analogous situation in most respects (302) (22). Similar lesions occur in riboflavin deficiency in the dog (292), mouse (27) and swine (235). The early observation by Spies, Vilter and Ashe (284) that certain ocular lesions frequently seen in pellagra improved with riboflavin therapy and the report by Park-Steen (234) that twilight blindness and other eye symptoms common in sprue, usually responded to riboflavin administration, were early evidences of the occurrence of this deficiency and these lesions in man.

Since riboflavin is a part of the respiratory apparatus (oxidation-reduction enzyme system) of the cell (17), one may speculate that this vascularization is a response to a gradually failing cellular respiration of the corneal epithelium. This is supported by the fact that vascularization is the usual response of the cornea to other kinds of chronic injury. In this case the handicap or injury is to the metabolic machinery of the cell in general. Therefore, the capillary ingrowth is diffuse and well developed before other morphological damage is evident.

Johnson and Eckhardt (156) report successes in the treatment of rosacea keratitis with riboflavin. This condition, of unknown etiology, is characterized by progressive recurrent focal injury, infiltration and necrosis of the cornea with capillary ingrowth and subsequent healing. Although distinguishable from the keratitis of uncomplicated riboflavin deficiency, it may represent a deficiency which is provoked or modified by an exciting agent or a constitutional factor. An alternative possibility would be that it represents the value of liberal riboflavin in healing processes of the cornea to injury of diverse causation. Improvement of some cases of early interstitial keratitis of syphilis (22) as a result of riboflavin therapy has been reported.

In addition to the early changes in the lids and cornea, Day and his collaborators (78) (79) (80) (81) have repeatedly produced cataracts in a majority of rats kept on riboflavin deficient diets longer than 70 days and finds mice, chickens and monkeys to respond likewise. Confirmation of these observations comes from Bourne and Pike (39) and others, but many laboratories (148) (26) for reasons still obscure, find cataracts only occasionally. Difference in colony susceptibility, degree of severity of the deficiency and the influence of the presence or absence of other factors in the ration are possible explanations. The earliest morphological changes in the lens are a proliferation of the epithelium and a breaking down of the fibers directly under the capsule. This progresses to complete dissolution of the fibers with formation of Morgagnean globules and conversion of the lens to an amorphous mass. Day (81) reports protection or arrest of the process with riboflavin.

Riboflavin deficiency has led to the development of dermatitis of varying degrees in most species studied (148) (263) (290) (152) (26) (248) (300) (114).

In rats on experimental rations, the distribution and appearance of the skin lesions led György (125) to differentiate clearly between riboflavin deficiency and that caused by a lack of another factor, pyridoxine (vitamin B_6). The dermatitis of the latter deficiency appears suddenly and is conspicuously severe on the paws and ears while the skin lesions of riboflavin deficiency are slow in development, generalized, and non-inflammatory in appearance. They are characterized by a dry greasy scaliness of the skin which tends to increase, a gradual loss of hair, most conspicuous in regions easy to scratch, and a subsequent replacement by a sparse fine curly coat of short abnormal hair. In time the skin becomes inelastic and greatly thickened. Lesions of the lips and mouth, similar to those occurring in man, are sometimes observed. Although skin lesions have been described by many investigators, careful histopathological descriptions and interpretations have not been reported.

We (335) (338) have not made sufficient study of the skin pathology to give a detailed description of the sequences in the development and repair of the deficiency lesions. We find that the initial responses are in the epidermis and its appendages. The vascular engorgement, so characteristic of pyridoxine deficiency, does not occur. The epidermis as a whole shows little change other than a moderate hyperkeratosis. In some locations there is slight hyperplasia of the epidermis, particularly of the snout and sides of the head, possibly related to scratching. Sebaceous glands, including the Meibomian glands of the eyelids become somewhat atrophic. There is an increased rate of shedding of hair which we believe to be the result of separation of the cornified anchoring cells from the epithelial sheaths. The outstanding and thus far, to us, distinctive feature of the deficiency is the effect upon regeneration of hair follicles and hair formation. In the late stage of the deficiency, regeneration of the hair follicles does not occur or is incomplete. Follicles engaged in hair formation during the establishment of the deficiency undergo atrophy and for a time continue to form imperfect hair. The atrophy is apparent in all parts of the hair follicle but is most evident in the matrix. The cuticular cells continue longest but undergo atypical cornification. Thus various degrees of retardation of hair production are found in a given area of skin: complete suppression, hair roots represented by loosely packed columns of cornified fusiform cells and hair roots consisting of medulla with imperfectly formed cortical substance. Sharply flexed or buckled hair follicles are common, presumably occasioned by the lack of support normally afforded by the forming hair shaft or root. In cross section, the hair roots are often oval or flat in outline. The microscopic appearances account satisfactorily for the gross appearances of the sparsely distributed hair. The gross impression of thickening of the skin may be accounted for by the persistence of many atrophic regenerated follicles because these may and often do extend to the depth of normal active follicles (i.e., to the muscle panniculus), and owing to their number, should affect the texture of the skin. In 48 hours after riboflavin therapy, there is marked restoration of normal appearances of the follicles and in 72 hours the epithelium of the follicle has assumed normal appearances. The matrix cells respond first.

On the dorsal surface of the tongue, the filiform papillae of the anterior portion exhibit an analogous retardation and defective formation of cornified cells. The pronounced effect of the deficiency upon hair formation as compared with the effect upon epidermal keratinizing sequences should eventually find explanation in the differences in composition of end products of the respective epitheliums.

An apparently similar skin condition frequently occurs in man, along with the ocular lesions of riboflavin deficiency (302) (264) (265) (300). This seborrheic dermatitis usually appears around the naso-labial folds but in some cases around the eyes and ears and occasionally in a generalized form. Like the eye lesions, the dermatitis responds to riboflavin therapy. This suggests that the hyperkeratosis follicularis frequently reported as occurring among the ill-nourished, especially in the Orient, might be related to riboflavin deficiency instead of A deficiency as previously concluded from rather inadequate evidence. Radhakrishna Rao (245) has recently studied the histopathology of this disease in India.

Conspicuous manifestations of riboflavin deficiency in man are reddening and desquamation of the lips and the development of fissures at the angles of the mouth, resembling perlache. The lesions begin with pallor at the angle of the mouth, followed by maceration and the formation of transverse fissures which are usually moist, with a light yellow exudate or crusted. A smooth tender magenta colored tongue, distinguishable from the scarlet atrophic tongue of nicotinic acid deficiency, contributes to the sore mouth. The human experimental studies of Sebrell and Butler (265) (264) and the subsequent observations of others (302) of the response of spontaneously occurring cases to riboflavin therapy make it evident that the angular stomatitis and facial seborrheic dermatitis reported by many earlier writers as observed in pellagra, sine pellagra, sprue, and among the ill-nourished groups in all parts of the world are due to riboflavin deficiency (264) (302) (169) (15). Diagnoses based on knowledge of the ocular, skin and mouth lesions indicate that this deficiency is not uncommon in the United States.

Riboflavin deficiency in the dog (338) and swine (235) and occasionally in the rat (27) leads to sudden collapse, coma and death, within a few hours and without apparent morphological cause for death. The onset is sudden and characterized by ataxia, weakness, inability to stand, and loss of deep reflexes of the limbs. The animal is fully conscious and without pain. The respiration is slow, becoming shallow and labored and finally failing. The condition is rapidly ameliorated by early parenteral riboflavin administration. The sudden collapse and rapid recovery indicate, as explanation, a failing chemical mechanism. This fits with the hypothesis that death is due to cellular asphyxia. Fields and Wise (105) suggest that an unexplained sudden human death they saw may have had a like cause. Sebrell and Onstott (263) reported the presence of "yellow livers" and degenerative changes in the central nervous system of these dogs. Others (292) confirmed the nervous symptoms and lesions but did not regularly find the fatty livers. Street, Cowgill and Zimmerman (292) found myelin degeneration of the peripheral nerves and in the posterior columns of the spinal cord which

increased in degree with the duration of the deficiency. Although riboflavin administration largely prevented these lesions, there remains a doubt that they are direct consequences of this deficiency.

Phillips and Engels (236) (237) reported degeneration of the sciatic nerves and degeneration in the spinal cords of chicks on rations low in riboflavin and pantothenic acid. They claimed riboflavin prevented the peripheral lesions, while the cord lesions were associated with pantothenic acid deficiency. A rapid and severe paralysis of the legs was characteristic of the acute deficiency, while a slowly developing disability of the feet (curled toe) and legs followed a chronic deficiency. Notched beaks (a lesion at the junction of the upper beak and the flesh) is also reported as a sign of riboflavin deficiency in chicks (172) (173).

Eggs failing to hatch for lack of riboflavin show abnormalities of the embryos among which are degeneration of the Wolffian bodies, deformed down, edema, and dwarf size. The defective down formation is interesting because it corresponds to the skin defects and abnormal hair formation in the rat.

We have found (338) no changes in the rat which can be regarded as other than the results of inanition, in the osseous system, skeletal musculature, cardiovascular system, respiratory tract, gastro-intestinal tract, genito-urinary tract, salivary glands, spleen, liver, pancreas, adrenals, thyroid glands, and parathyroid glands. The lacrimal glands are normal but the Harderian glands undergo very definite atrophy and fail to form their characteristic yellow pigment (a porphyrin derivative). The spleen is non-hematopoietic and without hemosiderosis, in contrast to vitamin A deficiency. Following replacement therapy it becomes actively hematopoietic. The liver cells are demonstrably atrophic, without glycogen or stored fat. In severe deficiencies, the central cells are vacuolated and occasionally degenerated. The testes cease to form spermatozoa. The adrenals are moderately atrophic.

Riboflavin deficient rats often become heavily infested with lice (128). The cause is unknown but apparently nonspecific since this may also occur in other deficiencies (27).

Pinkerton and Bessey (239) have found a striking increase in susceptibility of the rat to the rickettsiae of murine typhus as the animal progresses in riboflavin deficiency. Pure riboflavin has been found protective. Search for a similar increased susceptibility to other intracellular parasites and for an explanation of the phenomenon has been unsuccessful.

Nicotinic acid (p-p factor) deficiency. While recent events establish inadequate nicotinic acid as the principal dietary defect leading to clinical pellagra, they also confirm the long-standing belief that most pellagrins suffer from multiple deficiencies (94) (92) (284). This explains, in part, the great variations in symptoms and lesions occurring in this disease (282). A number of recent papers deal with the differentiation of individual symptoms and lesions based on specific treatment with the various pure vitamins (nicotinic acid, thiamin, riboflavin, etc.) and the relation of the responses to problems in diagnosis and treatment of pellagra (92) (286) (299) (341). There are no publications dealing with the pathology of uncomplicated nicotinic acid deficiency in either man or animals.

Pellagra and the corresponding disease in the dog (92) (144) (black tongue) thus far studied pathologically are, in all probability, the products of more than one deficiency. Since nicotinic acid cures certain of the ectodermal and entodermal lesions of these diseases, we may regard certain lesions of the skin and mucous membranes as the result of nicotinic acid deficiency. It is not clear at present whether the nervous lesions of pellagra and black tongue are specific late manifestations of nicotinic acid deficiency or a secondary nonspecific consequence of general disturbed tissue metabolism or an effect produced by the absence of some other essential nutritive factor (284) (282) (345) (342) (343). Myelin sheath degeneration of peripheral nerves and spinal cord tracts and complete degeneration of neurons have been described and are late manifestations. No specific feature in sequence or character of the degeneration of medullary sheath, nerve cells and axon are known. The prompt response to therapy indicates that most of the mental symptoms of pellagra have a physiological basis. The meager knowledge of the pathology of pellagra is summarized by Eddy and Dalldorf (92). Adequate descriptions of the gross appearances and distribution of the skin and mucous membrane lesions and all aspects of pellagra have been recently reviewed (286) (277) (266) (285) (18).

The lesions of the skin, mucous membranes and viscera of pellagra and black tongue have been described by Denton (83) (84). The important feature of his description of the skin lesions of pellagra (83) is that he assigns the primary rôle to changes in the corium consisting of edema and dissolution of collagen fibrils beneath the epidermis. Changes in the epidermis are regarded by him as secondary to those in the corium. Reparative proliferation of epidermis, vascular engorgement with ectasis and finally cicatrization of corium and epidermal atrophy complete the cycle. Lesions of the tongue, mouth, and esophagus, though more persistent, undergo similar sequences to those of the skin. Dalldorf (92) confirms Denton's findings and interpretation. The fact that repair of the pellagrous skin lesion is accompanied by cicatrization of the corium is strong support of Denton's conclusion that the initial lesion is in the superficial layer of the corium. Should similar results be obtained in experimental nicotinic acid deficiency in animals, an important lead would be established for physiological studies of the skin. Denton found no lesions of significance in the respiratory tract and in solid viscera. The mucosa of the colon is commonly involved in somewhat characteristic but not pathognomonic fashion, the outstanding features of which are atrophy and cystic dilatation of the crypts of Lieberkühn. Dalldorf (92) reviews briefly other conditions in which similar lesions of the colon are found. In addition to skin lesions, nicotinic acid deficiency in swine (experimental and spontaneous) leads to severe lesions of the intestinal tract. Studies of such material should lead to a better understanding of the pathology of this deficiency in both man and animals.

Denton's study of black tongue (84) was made in dogs from Goldberger's laboratory. His descriptions duplicate those made on pellagra material. He concludes his report with a statement of great significance, in urgent need of confirmation: "The distinctive lesions of pellagra and those of black tongue of

dogs appear to have their origin in a failure on the part of the organisms to maintain the specialized supporting tissues of epithelium in various situations."

PYRIDOXINE (VITAMIN B_6) DEFICIENCY. The emergence of vitamin B_6 (pyridoxine) from the heat stabile "vitamin B complex" was brought about by György and his collaborators (124) (125) (33). György defined vitamin B_6 as "that part of the vitamin B complex which is responsible for the cure of a specific dermatitis developed in young rats on the vitamin-free diet supplemented with vitamin B_1 and lactoflavin" (124). Birch, György and Harris (33) pointed out that vitamin B_6 deficiency is most characteristically a disease of the extremities and that the lesions are not truly "pellagra-like." They suggested that vitamin B_6 should be called "rat acrodynia factor" (125).

Studies of the pathology of pyridoxine deficiency have been almost wholly upon the rat, which is the only animal that shows the characteristic dermatitis, although recent reports indicate that it is also an essential for other animals, such as the chick (160) (143), dog (182) (293), pigeon (54) (57) and pig (326). The evidence that pyridoxine is a human requirement is meager and inconclusive (287).

Comprehensive studies of the pathology of pyridoxine deficiency in the rat have been reported by Sullivan and Nicholls (294) and Antopol and Unna (12). These two papers provide adequate historical information and a complete bibliography of the subject.

The skin lesions of pyridoxine deficiency in the rat are characteristic in distribution and gross appearance and can be distinguished from the dermatitis of riboflavin deficiency, pantothenic acid deficiency and egg white injury (preventable by biotin) (127). The paws, snout and ears are involved in an acute erythema and edema, followed by desquamation which may finally lead to ulceration, especially on the paws, where rubbing becomes a factor. Sullivan and Nicholls (294) claim that involvement of the ears occurs only when other factors of the "B complex" are absent.

We (338) regard involvement of the ears (pinnae) a consequence of pyridoxine deficiency and have seen the same gross and microscopic appearances here as on the paws. Antopol and Unna (12) regard the ear lesions as most characteristic and intimate that the failure of Sullivan and Nicholls (294) to obtain them was due to the presence of small amounts of pyridoxine in the preparation of the "filtrate factor" they used in their diet. All observers agree that the skin of the trunk is not demonstrably affected by the deficiency.

A detailed resume of the skin pathology hardly seems warranted because the changes which have been described and which we have studied carefully from our own material are not amenable to interpretation, or even intelligent rationalization, with reference to retardation or suppression of normal cutaneous processes; a state of affairs probably the result of our deficient knowledge of the physiology of the skin.

The microscopic changes in the epidermis are marked increase of thickness, the result of increased number and size of the cells of the layers, particularly of the prickle cell layer (acanthosis), the stratum granulosum, and the keratinized

or cornified layer (hyperkeratosis). Mitotic figures are common in the basal layer. The capillaries and vessels of the precapillary size in the corium are markedly engorged and there is usually some edema and infiltration, chiefly with mononuclear cells (monocytes and lymphoid cells). In advanced degrees of the deficiency there is evidence of intercellular edema of the epidermis. The thickened keratinized layer may separate as a cast. Ulceration is a late effect and bacterial growth in the keratinized layer, and trauma (the result of scratching) probably contribute to its production.

Sullivan and Nicholls (294) found that the hair follicles and sebaceous glands in the regions of the specific dermatitis remained intact until involved by secondary infection. They regard a general partial atrophy of sebaceous glands and hair follicles late in the deficiency as a result of prolonged inanition. Antopol and Unna (12) regard atrophy of hair follicles and sebaceous glands in regions of the specific dermatitis as a late primary effect, which is in agreement with opinions held by us (338). Antopol and Unna (12) studied all organs of the rat. When choline was present in adequate amounts, the liver showed no lesions. They describe, but do not regard as specific, cellular changes in the adrenal glands, the most prominent being atrophic changes in the reticular zone, with which we are familiar and in agreement as to the non-specific nature. They also found atrophy of the testes with aspermia which is probably not specific, since we and others have seen identical changes in the testes in vitamin A deficiency—(see *vitamin A deficiency*). Retardation of endochondral bone formation, as seen by Antopol and Unna (12) and us (338) is not different from that resulting from inanition, however produced.

We have seen no lesions in our pyridoxine deficient rats which we regard as peculiar to the deficiency in the following organs: heart, eye, thyroid, parathyroid, trachea, lungs, paraocular glands, salivary glands, stomach, intestines, liver, spleen, kidneys, pancreas, adrenals, ovary, testis, bone and bone marrow, lymph nodes, skeletal muscle, nervous system.

We have failed to find in our own material or in published descriptions any histological feature peculiar to pyridoxine deficiency. The early marked engorgement of blood vessels may be a clue to the initial physiologic defect. Of greatest significance as yet unrevealed is the symmetrical distribution involving the extremities. We (338) have found that light is not an important factor and that sympathetic denervation on one side did not produce a difference in the effect of the deficiency.

An outstanding premise for the future elucidation of the pathology of pyridoxine deficiency is that the dermatitis can be cured or prevented by administration of the so-called "essential fatty acids" (34) (257). Birch (34) suggests: "that the physiological function of vitamin B_6 is connected with the utilization of the unsaturated fatty acids."

Pyridoxine deficiency in the dog leads to a microcytic hypochromic anemia (182) (293). Street, Cowgill and Zimmerman (293) report that after nearly a year on diets free of pyridoxine, their dogs showed cardiac embarrassment and degenerative changes in the peripheral nerves and spinal cord. The cardiac

failure was prevented by pyridoxine concentrates, but mild nerve changes were also observed in the control dogs. In the chick (160) (143) there is retardation of growth and symptoms suggestive of lesions of the central nervous system Chick et al. (57) (58) report the occurrence of periodic convulsions (epileptiform fits) in swine and in rats maintained for long periods of time on pyridoxine-deficient diets. Protection with pure pyridoxine was reported. We have (338) occasionally observed similar convulsions in rats.

CHOLINE DEFICIENCY. The dietary importance and properties of choline logically include it among the substances being discussed in this paper. Low choline diets, especially when fat intake is high and protein low, lead to retardation of fat "turn over" through the liver and the development of fatty livers (28) (29). When the process is chronic, a diffuse fibrosis (cirrhosis) results; at least in the dog (55) and rat (36) (176). In addition to the development of fatty livers, acute choline deficiency in young rats leads to an early severe hemorrhagic condition of the kidneys and other tissues, resulting in a high mortality, but a surprising recovery is possible in animals which survive the few days of the acute period (120) (123). Cystine-rich diets produce or intensify the liver and kidney lesions while methionine, low-fat intake and choline have a mitigating effect (121). DuVigneaud (312) et al. have shown that methionine and choline are related in their action by both being methylating agents. Presumably the intensifying effect of cystine is due either to a competition for methyl groups or to improved growth with subsequent increased choline requirements. This whole subject has been recently reviewed by Best and Ridout (28) (29) and by Morgan (212) and Griffith (123). Griffith (121) (122), Christensen (60), Engel and Salmon (96), and György and Goldblatt (129) have described the lesions of acute choline deficiency in young rats as a hemorrhagic degeneration of the kidney, involution of the thymus, enlargement of the spleen and frequent hemorrhages into the myocardium, adrenal cortex, lymph nodes, eyes and lungs. The renal hemorrhages seem to appear first from arterioles at the periphery of the cortex beneath the capsule. In severe deficiencies glomeruli are incorporated in the hemorrhages, but it is not clear from descriptions that the glomerular arterioles become sources of hemorrhage. In acute fatal cases, extensive hemorrhages cause destruction of all elements of the renal parenchyma. Those animals which survive for several days or more show repair of the hemorrhagic necroses by organization and cicatrization. Although the mechanism underlying the hemorrhages is not yet clear, the function of acetylcholine as a neuromuscular mediator is suggestive of a neurovascular cause.

It seems probable that the lesions of cystine intoxication are analogous to those described above (90) because in the liver they are initiated by hemorrhagic necroses resembling those of eclampsia.

Jukes (161) (162) recently reported that choline is among the factors involved in the prevention of perosis, a condition characterized in chickens and turkeys by short, thick bones, notably of the tibia and tarsus, often with deformity and dislocation of the hock joint and slipping of the tendo-calcaneus (slipped tendon disease).

Rich and Hamilton (247) have reported the development of cirrhosis of the

liver of a type resembling Laennec's cirrhosis in man by maintaining rabbits on certain purified diets deficient in choline. Yeast prevented the lesion but the known vitamins were ineffective. Microscopically, the cirrhosis was diffuse and it was strikingly difficult to find necrotic liver cells in spite of the obvious reduction in parenchyma and the proliferation of connective tissue. The scarring appeared to be due not to repair of foci of necrosis involving many cells but rather a reaction to a more diffuse type of injury. They did not describe early stages of the liver lesions.

PANTOTHENIC ACID. Pantothenic acid was described, identified and finally synthesized by Williams and his various collaborators as an outcome of their interest in growth factors for yeast (323). It subsequently proved to be the much sought (filtrate factor) component of yeast and liver previously known to be necessary for growth and well being of the rat and for prevention of a dermatitis in chicks (170) (171) (251) (159) (307). It is an established nutritional essential for the dog (203) and mouse (256) and inconclusive evidence indicates many other species as well (210) (211). Pantothenic acid deficiency has not been reported in man perhaps because of its wide distribution in nature or a lack of knowledge of the signs and symptoms. The most significant lesion, preventable by pantothenic acid, that has been described, is a fatal hemorrhagic cortical necrosis of the adrenal glands in the rat (72) (210). According to Nelson (218) and Ashburn (14), congestion, hemorrhage, atrophy, focal and diffuse necrosis, cortical fat depletion and fibrosis occur as combined or independent lesions. The innermost zone (reticularis) is most severely affected, the damage becomes milder toward the periphery and the medulla is apparently not involved. This picture is reminiscent of the effects of certain toxins on the adrenal cortex. The sequence of events and interpretations of the lesions remain unsolved. The adrenal is reportedly not affected under like conditions in the mouse (171) (174). Other manifestations in this deficiency are alopecia, scaliness and thickening of the skin with eventual ulcerations, which occur about the nose and head and over the shoulders, flanks and abdomen of both rats and mice (171) (174) (248). Microscopically, the process appears to be a non-specific, hyperkeratotic, atrophic, desquamative dermatitis: a similar condition develops conspicuously on the the feet of the chick (250). Inflammation and encrustation of the nose, mouth and eyes with closure of the latter has been observed inconstantly in the rat and chick. Paralysis with degenerative changes in the sciatic nerve and cord has been reported by Lippincott and Morris (174) in the mouse and by Philips and Engel (237) in the chick.

Absence of spermatogenesis, narrowing of the epiphyseal cartilage and lack of bone growth are common reactions to several types of malnutrition. There is some controversy as to whether pantothenic acid is involved directly, indirectly, or not at all in the greying of the hair (achromotrichia) which has been observed in a variety of species when kept on experimental rations (210) (211) (110) (11) (131). It seems reasonably clear that more than one dietary defect or circumstance can lead to greying, among which may be included pantothenic acid deficiency. Apparently the process of melanin formation in the pigment cells of the skin and hair can be influenced by a variety of factors. Many of the

lesions described above as manifestations of pantothenic deficiency are common to general tissue disturbances; perhaps this is an added indication of the importance of this substance to all cells.

OTHER FACTORS. György, Goldblatt and Miller (126) find, occurring in some of their experiments on rats, an aplastic bone marrow with anemia, leukopenia and thrombocytopenia (panmyelophthisis). A similar condition has been described by Day et al. (82) in monkeys maintained on experimental diets. Protection is secured by yeast and the term vitamin M has been suggested until the responsible factor becomes known. Severe necrotic lesions of the gum and mucosa and peridontal tissues have been a feature in these deficient monkeys. Similar lesions have been described by others (304).

Pathological knowledge relative to several dietary factors, still not clearly differentiated, is so nebulous that discussion at this time seems unwise.

SUMMARY

The morphological manifestations of vitamin deficiencies may be grouped into four categories: 1, diffuse consequences expressive of inanition; 2, effects common to several deficiencies, especially degenerations of the nervous system and, with qualifications regarding distribution and fine details, lesions of the skin. Unknown factors, possibly absent from the experimental diets usually employed, may be responsible for some of the effects common to two or more of the deficiencies; 3, degenerative changes characteristic in kind and distribution, best illustrated by the cerebral lesions of B_1 deficiency and the degeneration of skeletal muscles and embryonal tissues in vitamin E deficiencies. The skin lesions of nicotinic acid, pyridoxine, pantothenic acid and riboflavin deficiencies and the ocular manifestations of the last may also be placed in this category. 4, Initial specific effects exhibited by striking changes in structural patterns, outstanding in relation to vitamins A, C and D.

In category 1 we include those effects which are similar to those of starvation as produced by inadequate amounts of a complete diet and we can list, with considerable assurance: *a*, retardation of growth including cessation of endochondral growth of bone; *b*, anemia as exhibited by decreased activity of the hematopoietic tissues; *c*, diffuse atrophy of skeletal muscle and various glandular organs, and *d*, retarded growth of hair.

In category 2 the effect of greatest importance is the degeneration of peripheral nerves and spinal cord tracts in the following deficiencies—thiamin, pyridoxine and riboflavin, and probably pantothenic acid, and possibly nicotinic acid. The nerve lesions reported as the result of vitamin E deficiency are probably secondary to degeneration of skeletal muscles. Mechanical factors, the result of a relative overgrowth of the central nervous system, cause the paralysis of vitamin A deficiency. With the exception of thiamin deficiency, the sequences of nerve degeneration have not been studied adequately. In this deficiency, myelin sheath degeneration follows degeneration of the axis cylinder as it progresses from the periphery toward the center.

The possibility of a deficiency directly affecting the formation and/or main-

tenance of myelin should be energetically explored because of its importance to the physiology and pathology of neurons.

In category 3 the outstanding degenerations which are characteristic in kind and distribution are those of vitamin E deficiency. The changes leading to early death of the fetus have at present descriptive value only because of our ignorance of the normal processes affected. The aspermia of vitamin E deficiency is permanent because the whole of the seminiferous epithelium degenerates, whereas in other deficiencies and in starvation inanition, the undifferentiated cells of the seminiferous epithelium survive. The muscle degeneration is the one consequence of the vitamin E deficiency which seems directly amenable to biochemical elucidation because the type of inactivity of the muscle secured by nerve section or by tendon cutting prevents its occurrence; this indicates that vitamin E may be directly concerned in the more or less known metabolic processes involved in muscle kinetics. If carbohydrate metabolism is at fault in the production of the muscle degeneration, reactions must be concerned unlike those in which thiamin operates because deficiency of the latter is not attended by skeletal muscle degeneration.

The nervous system lesions of thiamin deficiency presumably have origin in arrested carbohydrate metabolism; their localization may be an indication of relative metabolic rates of the neurons affected.

The hemorrhages of choline deficiency and vitamin K deficiency have yet to be elucidated, but indications point to a neurovascular mechanism for the former and to an unknown physiological rôle of prothrombin for the latter.

Category 4: vitamins A, C, and D deficiencies belong here. Each is characterized by important changes in structural patterns. The morphological sequences of each are easily followed and the reparative responses following replacement therapy are highly informative.

In vitamin A deficiency two apparently unrelated effects are outstanding; one the atrophy of many epitheliums with replacement by squamous keratinizing epithelium which occurs regardless of age; the other—of importance only during the period of rapid growth—a degree of retardation of skeletal growth in relation to the growth of the central nervous system, which results in mechanical compression of brain, spinal cord, and nerve roots. The epithelial effect may be manipulated, by means of periods of replacement therapy, for the study of fundamental problems in cytology, particularly in relation to such shifts in physiology as the morphological changes we have described indicate. The continuously growing incisor teeth of rodents provide opportunity for further studies upon the differentiation of cells through subjecting the mesenchymal tissue to intermittent organizing influences from the enamel organ as it responds to periods of vitamin A deficiency and recovery.

Understanding of the mechanism of the bone destruction caused by greatly excessive amounts of vitamin A should be achieved by means of coördinated biochemical and pathological studies, such as we now have in progress. The solution should give premises of value for the consideration of various aspects of bone physiology and pathology, and possibly a clue to the biochemical rôle of vitamin A.

The structural changes in ascorbic acid deficiency are the result of the failure of formation and maintenance of intercellular materials. Specifically this narrows down to problems of tissue collagen formation and its maintenance. We believe that our present knowledge of intercellular physiology can be extended considerably by careful studies of the occurrences which accompany intermittently induced vitamin C deficiency, as exhibited in regions of normal growth or in experimentally induced repair processes. Experimental vitamin deficiency pathology as well as human pathology and physiology provide many premises for the belief that collagen formation may be a reversible process. Vitamin C deficiency experimentation affords the best means of attacking this and other problems of supporting tissues suggested by their ever-changing pattern accompanying growth.

Vitamin D deficiency structural changes are explainable by the simultaneous occurrences of two conditions, one the retardation or suppression of normal growth sequences in epiphyseal cartilage, the other failure in calcification of bone and cartilage matrices. Apparently the failure of mature cartilage cells to degenerate and failure of matrices to calcify are results of a common factor—deficiency in calcium and/or phosphorus ions in the blood and extra-cellular materials. Nevertheless, the chemistries involved may be very different; an enzyme system in cartilage cells and critical precipitating levels in matrices.

The phenomena of hypervitaminosis D indicate that vitamin D in addition to increasing absorption of calcium and phosphorus from the intestines, is responsible for establishing a system in the blood plasma which withdraws calcium and phosphorus from available sources, including the bones. The demand for these elements and the ability to hold them in solution seem to have quantitative relations with the amount of vitamin D introduced. The deposition of calcium salts in soft tissues as the blood calcium level falls following a brief period of hypervitaminosis D has not been carefully studied in regard to the exact locations of deposition and relations to intercellular materials.

A few considerations should be kept in mind in planning vitamin deficiency experiments either for the purpose of characterization of a deficiency or for the elucidation of normal processes of growth and function. One is that the dietary regimen should be optimal in every respect excepting the content of the vitamin concerned, because other conditions which retard the general metabolic rate may decrease that vitamin requirement. A second is that the consequences of a complete deficiency may be so severe as to prevent survival of the animal for a long enough period for the development of distinctive functional and structural changes, at least in demonstrable forms. Acute thiamin deficiency affords an example.

A third is that the more rapid the growth rate the greater is the effect upon processes concerned in synthesis of structural materials. Ascorbic acid deficiency and vitamin A deficiency are examples.

A fourth is that there are important species variations, ranging from complete independence of a vitamin, as is the case of most vertebrates for ascorbic acid, to minor differences such as the failure of most laboratory animals to develop

the characteristic skin lesions of pyridoxine deficiency exhibited by the rat; the persistence of spermatogenesis in the mouse in vitamin E deficiency, the independence of the rat for vitamin D when provided adequate Ca and P in the food and presumably variations in skeletal effects of vitamin A deficiency as suggested by the few studies on the rat, dog and cow.

The usefulness of morphological studies of vitamin deficiency conditions extends in several directions. There are obvious applications to problems in general biology. For the physiologist and biochemist, the great value is the information to be obtained suggestive of the kind of metabolisms in which a vitamin is involved. We know, by way of problems amenable to biochemical attack, that ascorbic acid is necessary for collagen formation; that vitamin A is necessary for the functions of various epitheliums though not for their growth and survival and also for bone growth in an as yet undetermined specific manner and for vision; that vitamin E is necessary for skeletal muscle metabolism and for survival of early embryonic tissues indicative of its participation in fundamental vital processes.

The correlation of chemical studies of blood, tissues and excreta with the structural changes in bone produced by avitaminoses A, C and D and hypervitaminoses A and D and by parathormone experimentation offers promise of achieving understanding of many problems of bone physiology and pathology.

There is great need, in neuropathology, for information about myelin, its formation, its maintenance, and its physiological rôle. Extended studies of the several vitamin deficiencies apparently concerned in the causation of myelin degeneration are indicated.

The various disorders of the skin and its appendages caused by vitamin deficiencies, notably those of the B complex, suggest possibilities of obtaining fragments of information about skin physiology. Present knowledge is suggestive that in the same animal there are local differences in skin physiology.

Another field of promise is the careful study of the bone marrow for specific changes accompanying deficient formation of blood cells.

We conclude with the expression of opinions that much desirable information is available through the study of tissues prepared by simple techniques and that searching cytological studies involving more difficult techniques offer very great promise.

The association of biochemical systems with structural details within cells is a biological goal of greatest importance. Success with a few systems in which vitamins are operative does not seem impossible of achievement and should carry us far toward a conception of the anatomy of life.

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